

FY03
NIH Extramural Support
in Bacteriology Research

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Grant: 1R01AA013745-01A1
Program Director: RUSSO, DENISE A.
Principal Investigator: KESHAVARZIAN, ALI
Title: Alcohol, iNOS upregulation, leaky gut & liver disease
Institution: RUSH UNIVERSITY MEDICAL CENTER CHICAGO, IL
Project Period: 2003/05/01-2008/04/30

DESCRIPTION (provided by applicant): Clinically significant alcoholic (A) liver damage (LD), secondary to a hepatic necroinflammatory cascade (HNIC), occurs only in a subset of alcoholics. Hence, factors other than ethanol (E) must be involved. Hypothesis: The key cofactor for ALD is a breakdown of gut barrier integrity ("leaky gut") due to chronic E use, which allows intestinal endotoxin to reach the liver & initiate a HNIC; this leakiness is due to cytoskeletal instability caused by oxidation of cytoskeletal proteins which is elicited by E-induced gut iNOS upregulation & nitric oxide (NO) overproduction. We found: 1 } in man, gut leakiness in alcoholics with LD but not in those without LD or in nonalcoholics with LD; 2 } in rats, E-induced leaky gut is associated with LD; reversal of gut leakiness attenuates LD; 3 } in intestinal monolayers, E-induced iNOS upregulation causes cytoskeletal & barrier disruption. We will continue to use this successful translational approach (monolayers, rats & man) to test our current hypotheses. Aims: (1) To see if, in a larger sample, a leaky gut: a) occurs only in alcoholics with LD & precedes cirrhosis b) persists during abstinence & after liver transplant for ALD, c) correlates quantitatively with LD severity, d) is associated with NO overproduction & HNIC, e) is more pronounced in females. We predict that gut leakiness (excess urinary lactulose, mannitol & sucralose levels after oral sugar load): i) is seen only in alcoholics with LD, precedes cirrhosis; ii) correlates with severity of LD (clinical parameters, liver enzymes); iii) is associated with NO overproduction (gut mucosal NO), serum endotoxin & HNIC (high neopterin/cytokines). (2) To see if, in rats, prevention of E-induced leaky gut also prevents E-induced LD & if a hyperactive, NO pathway is involved. We predict that in E-fed rats with LD: i) leaky gut, endotoxemia, HNIC, upregulation of intestinal iNOS, NO overproduction & oxidation of actin & tubulin occurs; ii) preventing gut leakiness (by oats, iNOS inhibitors or Arginine) prevents LD. (3) To see, using monolayers of wild type ((inhibitors) & transfected cells, if E-induced iNOS upregulation & its consequences (assessed by cytoskeletal oxidation/disarray & barrier disruption) are mediated by NF-kappaB activation. We predict i) E activates NF-kappaB by degrading IkappaBalpha; ii) preventing NF-kappaB activation prevents E-induced iNOS upregulation & its consequences. Significance: Showing that ALD requires a leaky gut, & that NO & cytoskeletal pathways are involved, could 1) identify drinkers at risk for LD (sugar test); 2) lead to therapies to prevent LD in those drinkers unable to abstain.

Grant: 1R01AA014243-01
Program Director: PUROHIT, VISHNU
Principal Investigator: WHEELER, MICHAEL D BS
Title: Acute Ethanol-Induced Innate Immune Response in Liver
Institution: UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC
HILL
Project Period: 2003/04/01-2008/03/31

DESCRIPTION (provided by applicant): The involvement of endotoxin in early alcohol-induced liver injury is well established; supporting the hypothesis, that pathogenesis involves aspects of innate immunity and inflammatory mediators. A number of reports have clearly demonstrated that Kupffer cells play a critical role in the pathogenesis due to ethanol. Specifically, it was shown that mice deficient in the endotoxin receptor CD14, which is primarily expressed on Kupffer cells, were resistant to chronic alcohol-induced liver injury. These data suggest that CD14 signaling may be a critical component in alcohol-related liver injury. The overall hypothesis is that LPS from the gut activates Kupffer cells causing an increase in oxidant production and subsequent TNF-alpha release. This hypothesis is strongly supported by a number of studies using gene therapy, knockout and transgenic animals, as well as an in vivo mouse intragastric ethanol-feeding model. Despite a great amount of new information regarding the role of endotoxin in ethanol-induced liver injury, critical gaps still exist in our understanding. For example, the signaling mechanisms involved in LPS- induced oxidant production, the regulation of CD14 and related signaling components in pathogenesis, and the molecular mechanisms determining gender- related differences in injury remain unknown. The purpose of this application is to address the following underlying hypotheses: 1. PI3 kinase mediates LPS-induced NADPH oxidase generation of superoxide in Kupffer cells. 2. Oxidant-sensitive transcription factors NF-kappaB and AP-1 regulate CD14 expression following acute ethanol administration. 3. Gender differences in regulation of innate immune response and transcription factor activation are key to increased susceptibility to ethanol-induced pathogenesis in females. The aims below will use gene delivery techniques and knockout mouse technology to address these critical questions related to roles of LPS, PI3 kinase activation, and CD14 expression in both acute ethanol and chronic ethanol toxicity.

Grant: 1R21AA014505-01
Program Director: ROACH, DEIDRA
Principal Investigator: ROSENBERG, ABRAHAM PHD
Title: HIV activation in alcohol-aided *G. vaginalis* virulence
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 2003/09/30-2006/08/31

DESCRIPTION (provided by applicant): The objective of this exploratory research application is to open an innovative approach towards understanding the role of alcohol-enhanced co-infective agent virulence factors in AIDS promotion in women. The application proposes to test the novel hypothesis that *Gardnerella vaginalis* is a major opportunistic HIV co-infection agent for women that features alcohol enhanced activity of its important virulence factor, i.e sialidase. The degree of sialylation is known to inversely affect the extent of replication and the infectivities of human HIV and other primate lentiviruses. Sialidase is an enzyme that has been shown to remove sialic acid from highly sialylated virion envelope gp120 and infectable target host cell CD4/chemokine receptors and, in so doing, dramatically escalate their high affinity interaction, virus binding, entry into the host cell, and viral replication. Sialidase activity is enhanced many-fold by alcohol levels that are achieved during binge drinking. A corollary of this hypothesis is that prophylaxis with sialidase inhibitors will reduce the risk of AIDS promotion in alcohol-abusing women co-infected with *Gardnerella vaginalis* and HIV. The exploratory R21 application proposes to test the following sub-hypotheses: 1) that *Gardnerella* sialidase will effectively remove sialic acid from gp120 and CD4; 2) that alcohol enhances the rate and extent of this de-sialylation of gp120 in the HIV viral coat and CD4 on CD4+ target cells such as T lymphoid, monocytoid, and peripheral blood mononuclear cells; 3) that de-sialylation of gp120 and CD4 promotes HIV entry and replication in the target cell; 4) that sialidase inhibitors prevent enhancement by *Gardnerella vaginalis* sialidase of viral entry and replication. The major recognized opportunistic infectious agents in the course of AIDS, namely the Eubacteriales pneumococcus, streptococcus, and bacteriodes; the protist *Trypanosoma cruzi*, and the fungus *pneumocystis carinii* all express sialidase as a major virulence factor. The present application makes the innovative connection of co-infective microbial sialidase as an AIDS-promoting bacterial virulence factor and that of *Gardnerella* sialidase relatable specifically to women. The stimulatory effect of co-infection with the *Gardnerella vaginalis* microorganism is known to advance AIDS progression in women, but the mechanism needs to be elucidated. This exploratory research application may help advance understanding of this phenomenon.

Grant: 1R03AG022185-01
Program Director: DUTTA, CHHANDA
Principal Investigator: FLYNN, MICHAEL G
Title: Aging, physical activity and toll-like receptor 4.
Institution: PURDUE UNIVERSITY WEST LAFAYETTE WEST LAFAYETTE, IN
Project Period: 2003/05/01-2005/04/30

Interleukin-1beta (IL-1beta), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha), play an important role in the pathogenesis of many "age" and "inactivity"-related diseases, but the mechanism for age- and physical activity-induced differences in inflammatory cytokine production has not been elucidated. Toll-like receptor 4 (TLR4), in concert with CD14, transduces the lipopolysaccharide (LPS) signal, but no researchers have documented the effects of either aging or physical activity on TLR4. The broad objective of this application is to examine whether age or physical activity level influence the cell-surface expression of TLR4 and to determine to what extent these changes are related to differences in inflammatory cytokine production. The primary aims of the proposed research will be to assess the influence of age and physical activity level on TLR4 expression and to assess concomitant changes in LPS-stimulated inflammatory cytokine production, single-cell cytokine production and MAP kinase activation. Heat shock proteins (HSP60/72) are released from muscle following exercise and are known to bind and activate TLR4. Thus, we will also endeavor to determine the role heat shock proteins play in these relationships. It is hypothesized that both younger and physically active subjects will have both lower cell-surface expression of TLR4 and cytokine production than their older and more sedentary counterparts. We also hypothesize that LPS-stimulated MAP kinase activation will be proportional to cell-surface expression of TLR4, but will not be different between groups when alternative stimulation pathways are engaged (anti CD40). We will recruit 16 subjects into each of four groups: 18-35 years of age, physically inactive; 18-35, physically active; 65-85, physically inactive and 65-85, physically active. Blood samples will be obtained from subjects who have not exercised for 72 hours, following 24 h dietary control. Cell-surface expression of TLR4/CD14 and single-cell cytokine production (flow cytometry), in vitro cytokine stimulation (LPS, HSP72 and HSP60) and MAP kinase activation will be determined. A 2 x 2 factorial design with age (18-35 and 65-85) and physical activity level (active and inactive) as the two experimental factors, will be employed. These experiments will help to determine whether age and physical activity related differences in cytokine production are linked to TLR4 and whether heat shock proteins play a role in TLR4 regulation.

Grant: 1P01AI055637-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: NORGARD, MICHAEL V
Title: Molecular Biology of Francisella tularensis Virulence
Institution: UNIVERSITY OF TEXAS SW MED DALLAS, TX
CTR/DALLAS
Project Period: 2003/09/30-2008/02/28

DESCRIPTION (provided by applicant): This P01 Project is in response to NIH Guide Notice NOT-AI-02-023, calling for research to improve the prevention, detection, diagnosis, and treatment of diseases caused by potential bioterrorism agents. Francisella tularensis is a Class A biothreat that is poorly understood at virtually every level, largely due to its zoonotic origin and extreme biohazardous nature. Recently, the Working Group on Civilian Defense highlighted the need for tularemia research in a number of basic areas. This P01 project addresses many of these major research gaps by bringing together a talented group of investigators with diverse, but complementary, expertise and with shared interests in the molecular basis of bacterial pathogenesis. The principal theme of this Program is to study how F. tularensis models its outer cell surface and immediate external environment to carry out its complex parasitic strategy. Project 1 (Dr. Simon Daefler) will explore the intracellular trafficking pathway of the F. tularensis-containing vacuole with emphasis on characterizing candidate virulence factors that ostensibly block fusion of the bacteria-containing endosomes with lysosomes. Project 2 (Dr. Eric Ransen) will investigate iron acquisition by F. tularensis and its influence on virulence expression. Project 3 (Dr. Kevin McIver) will analyze products secreted by F. tularensis into the extracellular environment that may represent toxins and other virulence factors. Project 4 (Drs. Michael Norgard and Kayla Ragman) will identify and characterize the outer membrane proteins and lipoproteins of F. tularensis. Project 5 (Dr. Vanessa Sperandio) will explore the genetics of virulence expression by F. tularensis and develop new genetic systems for manipulating F. tularensis. A particular strength of this Program is its focus on Schu4, a strain of F. tularensis that is highly infectious for humans. Progress in the individual projects will be enhanced substantially by the sharing of new developments, reagents, and specialized facilities and technologies. The combined studies will further our basic and working knowledge of F. tularensis pathogenesis that likely will lead to new diagnostic, vaccine, and intervention modalities for tularemia.

Grant:	1P01AI056013-01	
Program Director:	BAKER, PHILLIP J.	
Principal Investigator:	MANCHESTER, MARIANNE	PHD
Title:	Multivalent display of anthrax toxin inhibitors	
Institution:	SCRIPPS RESEARCH INSTITUTE	LA JOLLA, CA
Project Period:	2003/09/30-2008/01/31	

Bacillus anthracis produces a toxin that causes cell death and mortality in infected individuals. Even if the bacterial infection itself is controlled by antibiotics, an infected person often dies because the anthrax toxin (AT) produced in vivo by B. anthracis binds to human cells, is endocytosed, and kills the cells. Therefore development of specific antitoxins that prevent AT from binding to human cells is necessary for effectively treating anthrax exposure. This is the goal of this Program Project grant. The groups of J. Young and J. Collier have discovered and characterized the interaction between AT and its cellular receptors. This work fuels the discovery and development of novel peptide-based anthrax anti-toxins (Projects 1 and 2). Here we propose to display the inhibitory peptides and soluble AT receptor-based proteins in a multivalent form using the plant virus particle cowpea mosaic virus (CPMV) or the insect virus Flock house virus (FHV) as display platforms (Projects 3-5). These multivalent platforms will be produced and tested for their efficacy as antitoxins both in vitro and in vivo. These viruses are heat and acid-stable and are easy to produce in large quantities. Both viruses have been very well characterized genetically, biochemically and structurally and can either be engineered, or chemically modified, to display multiple copies of foreign immunogenic or antitoxin peptide sequences on their surface. The Manchester group (project 3) has previously shown that a virus-cellular receptor interaction can be efficiently blocked, both in vitro and in vivo, by the multivalent display of an inhibitory peptide on CPMV. Using this exciting result as a proof of concept, we will test the hypothesis that multivalent display of AT inhibitory peptides on CPMV and FHV will lead to the creation of a panel of new and effective anthrax antitoxins and vaccine reagents.

Grant: 1P01AI056293-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: NATHAN, CARL F MD INTERNAL
MED:IMMUNOLOGY
Title: MACROPHAGES, DENDRITIC CELLS AND PATHOGENS
Institution: WEILL MEDICAL COLLEGE OF CORNELL NEW YORK, NY
UNIV
Project Period: 2003/09/15-2008/02/29

DESCRIPTION: The purpose of this Program Project is to enhance basic knowledge at the molecular level of host-pathogen relationships, focusing on macrophages, dendritic cells and their products, with respect to 3 bacterial pathogens, their products and their mechanisms of evasion or repair-- Mycobacterium tuberculosis (Mtb), Bacillus anthracis (Ba) and Yersinia pestis (Yp)-- and to use this knowledge to develop translational approaches to novel chemotherapeutics and vaccines. Mtb, Ba and Yp were chosen for study because (i) they allow us to compare and contrast different consequences of bacterial interaction with macrophages and DC; (ii) in each case, those interactions--whether positive or negative---are critical to the pathogenesis of the disease; (iii) bronchopulmonary macrophages and DC often play a key role in the initial encounter of the host with these pathogens; and (iv) these pathogens use diverse mechanisms to evade or repair the antimicrobial lesions inflicted on them by host macrophages and DC. Specifically, this Program explores how human bronchopulmonary macrophages respond to Ba spores and toxins at the level of gene expression; how we can develop new inhibitors of enzymes within Mtb and Yp that are critical to the pathogenesis of their infections, namely, enzymes that degrade oxidized or nitrosated proteins and synthesize siderophores; and how we can use gene therapy vectors to target exogenous antigen into the appropriate compartments of DC to induce an effective vaccine against Yp while simultaneously providing for short term passive immunity. Cores provide for BSL3 wet lab and mouse work; microarray analysis of gene expression; computation and comparison of gene expression results; and high throughput screening of chemical libraries.

Grant: 1P01AI056320-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: METZGER, DENNIS W
Title: Mucosal Immunopathogenesis of *Francisella Tularensis*
Institution: ALBANY MEDICAL COLLEGE OF UNION ALBANY, NY
UNIV
Project Period: 2003/09/30-2008/03/31

DESCRIPTION (provided by applicant): *Francisella tularensis* is considered a Category A agent by the NIAID because of its extreme infectivity, ease of dissemination, and substantial capacity to cause illness and death. The "characterization of innate and adaptive immune responses that occur after initial exposure to *F. tularensis*" has been identified as one of the priorities of NIAID's Counter-Bioterrorism Research Agenda. The pneumonic form of tularemia is the deadliest form of disease and the form most likely to be used by bioterrorists, yet the great majority of research against this organism has focused on systemic infection rather than pulmonary tularemia. The overriding hypothesis of the Program Project is that the pathogenesis of *F. tularensis* in the respiratory tract is unique and that distinct mechanisms of mucosal-specific immunity are required for protection against pneumonic tularemia. The Program Project brings together a diverse group of individuals with particular expertise in the fields of microbiology, cell biology, and mucosal immunology who will explore in an integrated fashion, the immune response to *F. tularensis*. The four subprojects will: 1) Define the immunobiology of *F. tularensis*-macrophage interactions and determine the influence of macrophage activation state on killing of the organism, antigen presentation, and elaboration of inflammatory cytokines. 2) Examine the role of *F. tularensis* pattern recognition by the innate immune system in fostering lung inflammation. 3) Determine the importance of mucosal immune mechanisms in protection against pneumonic tularemia and develop novel strategies for induction of protective respiratory immunity. 4) Develop *F. tularensis* mutants to investigate the pathogenic consequences of the organism's interactions with macrophages. The overall goal of the Project is to characterize the association of *F. tularensis* with macrophages, particularly alveolar macrophages, and develop approaches for effective protection at mucosal surfaces. The results of these studies will ultimately be used to evaluate new mucosal vaccination strategies and new vaccine candidates against human respiratory infection with *F. tularensis*.

Grant: 2R01AI008619-34
Program Director: PERDUE, SAMUEL S.
Principal Investigator: COHEN, STANLEY N
Title: Mechanisms of Antibiotic Resistance in Bacteria
Institution: STANFORD UNIVERSITY STANFORD, CA
Project Period: 1991/07/01-2007/11/30

DESCRIPTION (provided by applicant): The long-term goal of this project is to understand the processes involved in the evolution of antibiotic resistance genes and their dissemination to populations of pathogenic bacteria. There is evidence that at least some antibiotic resistance genes have originated in streptomycetes--developmentally, morphologically, and biochemically complex antibiotic-producing bacteria that are chromosomally resistant to the antimicrobials they synthesize, and which can transfer segments of chromosomal DNA to both circular and linear plasmids. The proposed research, which continues and extends an ongoing multifaceted effort, is aimed at: 1) elucidating the role of pleiotropic and pathway-specific regulators of morphological differentiation, antibiotic biosynthesis, and antibiotic resistance in *S. coelicolor* and *S. lividans* on a genome-wide basis, 2) identifying, characterizing, and elucidating the mechanism of action of, and cellular targets of, small regulatory RNAs (sRNAs) of *Streptomyces*, and 3) elucidating mechanisms involved in the evolution and propagation of linear plasmids. The research will employ a combination of genetic and biochemical approaches, including mutational analysis of--and overexpression of--genes in regulatory pathways, DNA microarray studies of expression of *Streptomyces* genes and small intergenic RNAs, protein purification and analysis, and genetic analysis of linear extrachromosomal replicons. Collectively, the proposed studies will provide information that potentially will be useful in limiting the natural spread of antibiotic resistance genes, in developing antimicrobials that circumvent existing resistance mechanisms, and in combating antibiotic resistance that may be introduced intentionally, as acts of bioterrorism, into populations of pathogenic or toxigenic bacteria.

Grant: 2R01AI011709-24A2
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: CLARK, VIRGINIA L PHD
MICROBIOLOGY:BACTERIOLOGY
Title: Function of gonococcal anaerobically induced proteins
Institution: UNIVERSITY OF ROCHESTER ROCHESTER, NY
Project Period: 1977/02/01-2008/02/28

DESCRIPTION (provided by applicant): *Neisseria gonorrhoeae* is the etiologic agent of gonorrhea, one of the most prevalent infectious diseases in the U.S. Complications of gonorrhea include pelvic inflammatory disease (PID), the leading cause of sterility in females in this country. Despite the ability to effectively treat gonorrhea with antibiotics, the incidence of this disease remains high, suggesting that the most likely means of controlling the epidemic in the U.S. will be by vaccination. Elimination of gonorrhea will require an understanding of the host immune response to and pathogenesis of *N. gonorrhoeae*. One of the central themes of microbial pathogenesis is that the pathogen may express virulence determinants in vivo that are not expressed in vitro. We have been investigating the regulation of gonococcal gene expression by anaerobiosis, an environmental condition that this pathogen is likely to encounter in vivo in females. We have identified two anaerobically induced genes, *ani A* and *norB*, that are induced anaerobically and encode nitrite reductase and nitric oxide reductase, respectively. Both of these proteins are required for anaerobic growth and their presence means that gonococci can both produce and degrade nitric oxide (NO), an important modulator of the host innate immune response. We propose 1) to perform genetic and biochemical analyses of anaerobically regulated genes; 2) to determine the role of anaerobically induced and repressed genes in gonococcal invasion via the lutropin receptor; 3) to determine steady state NO concentrations during gonococcal denitrification and the effect of environmental parameters; and 4) to determine if gonococcal production/degradation of NO and/or steady-state NO levels down regulate cytokine expression and activation of soluble guanylyl cyclase. The successful completion of these aims should provide important new information on the function of the denitrification pathway and its role in pathogenesis. In addition, significant new insights into the mechanism of gonococcal suppression of the innate immune response may be attained.

Grant: 2R01AI016892-24
Program Director: PARK, EUN-CHUNG
Principal Investigator: SAUER, ROBERT T PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: Bacterial protein tagging, degradation & ribosome rescue
Institution: MASSACHUSETTS INSTITUTE OF CAMBRIDGE, MA
TECHNOLOGY
Project Period: 1980/04/01-2008/03/31

DESCRIPTION (provided by applicant): The objective of this research program is to understand the SsrA (tmRNA) system of E. coli, to probe its roles in ribosome rescue, protein tagging, and other cellular processes, and to determine how bacterial and phage proteins bearing ssrA tags or other degradation signals are recognized and degraded by bacterial proteases. We will probe the mRNA and/or protein determinants that induce SsrA tagging, study the biological function of full-length protein tagging, and determine which macromolecular factors associate with SsrA RNA during different parts of the tmRNA cycle. We will also study the structure, function, and substrate-binding specificity of ClpXP, the major protease that degrades ssrA-tagged proteins, and SspB, a modulatory factor that collaborates with ClpXP to enhance degradation of ssrA-tagged proteins. Repressors and other important regulatory factors are frequent targets of ClpXP degradation. Understanding SsrA function, ribosome rescue, and protein degradation are key goals of basic research in molecular and structural biology, with potential applications in medicine, biotechnology, and the design of novel proteins and regulatory circuits. For example, the SsrA system is required for the infectivity of bacterial pathogens and allows bacteria to withstand higher doses of antibiotics that inhibit protein synthesis. Analysis of the sites of SsrA tagging reveals locations of ribosome distress, providing a unique glimpse of the molecular events that hinder or impede protein biosynthesis. Such information could permit improved expression of recombinant proteins. ClpX serves both as the regulatory subunit of the ClpXP protease and as an AAA+ family disassembly chaperone. A detailed knowledge of ClpX-substrate recognition could allow the design of enzymes with altered specificity for use as tools in discovery research. ClpX, ClpXP, and SspB also serve as models in which to understand molecular mechanisms that have been conserved from bacteria to humans and adapted by all cells in processes ranging from protein degradation to membrane fusion.

Grant: 2R01AI017986-22
Program Director: PERDUE, SAMUEL S.
Principal Investigator: PRATT, REX F PHD
Title: Beta-Lactamases and DD-Peptidases--Active Site Chemistry
Institution: WESLEYAN UNIVERSITY MIDDLETOWN, CT
Project Period: 1982/09/30-2008/01/31

DESCRIPTION (provided by applicant): Bacterial resistance to beta -lactam antibiotics continues to become more prevalent and more clinically important. A large part of the resistance can be understood and investigated experimentally in terms of the chemistry of the interactions of a -lactam antibiotics with the active sites of two groups of bacterial enzymes, the beta -lactamases on one hand, which catalyze the hydrolysis of the antibiotics, and the D-alanyl-D-alanine transpeptidase/carboxypeptidases on the other, which catalyze the synthesis and maintenance of the peptide cross-links of bacterial cell walls, and which are inhibited by beta -lactam antibiotics. There is now good reason to believe that all of these beta -lactam binding sites have much in common. An understanding of the structure and function of these sites and of the relationship between them is fundamental to future antibiotics design - both beta -lactam and otherwise. The object of the proposed research is to explore further the chemical functionality and the substrate binding properties of a series of these active sites, using a number of modified substrates, novel inhibitors and potential effectors. Particular focus will be on the development of ligands, substrate and inhibitors, for the transpeptidases which, to date, have exhibited little in vivo activity except with beta -lactams. This goal will be accomplished by a combination of rational design, combinatorial chemistry, and target-accelerated methods. Crystal structures will be used in conjunction with molecular modeling to interpret the results obtained and apply them to further ligand design. These studies will lead to new insight into the chemistry of beta -lactamase and transpeptidase active sites, and thus to new directions in antibiotic design.

Grant: 2R01AI020016-17A2
Program Director: RUBIN, FRAN A.
Principal Investigator: CLEARY, PAUL P
Title: Streptococcal Inactivator in Human Chemoattractants
Institution: UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN
Project Period: 1997/09/01-2006/12/31

DESCRIPTION (provided by applicant): This project is an attempt to understand the mechanism by which group A streptococcus modulates host defenses. These streptococci have evolved proteins including the C5a peptidase that destroys C5a, an important early signal that attracts protective phagocytes to infected tissue. The recent discovery that the peptidase is also a fibronectin and cell-binding protein has become a significant new focus. Experiments are planned to evaluate the importance of these activities, relative to peptidase activity in virulence using intranasal and subdermal mouse models of infection. Recognition that penicillin often fails to eliminate group A streptococci from throats of ill children and the recent resurgence of serious complications associated with streptococcal infections, has prompted an intense effort to develop preventive vaccines by industry and NIH. The C5a peptidase is a major focus of vaccine development, increasing the need to learn more about its role in virulence, and its impact on host defenses. New data indicates that the C5a peptide required for successful colonization and persistence of streptococci in nasal associated lymphoid tissue.

Grant: 2R01AI020148-21
Program Director: SCHMITT, CLARE K.
Principal Investigator: O'BRIEN, ALISON D PHD
MICROBIOLOGY:MICROBIO
OGY-UNSPEC
Title: Pathogenicity of Shiga Toxin-Producing E. coli
Institution: HENRY M. JACKSON FDN FOR THE ADV ROCKVILLE, MD
MIL/MED
Project Period: 1983/08/01-2008/02/29

DESCRIPTION (provided by applicant): Enterohemorrhagic Escherichia coli (EHEC) O157:H7 belongs to a subset of Shiga toxin-producing E coli (STEC) that makes Shiga toxin (Stx) type 1 or type 2 (or a variant thereof), or both toxins, harbors pO157 (or a related plasmid) and expresses the adhesin intimin. Intimin is the product of the eae gene that is contained within a -43 kb pathogenicity island called the locus of enterocyte effacement or LEE. EHEC O157:H7 is the most common cause of bloody diarrhea (also called hemorrhagic colitis or HC) in the U.S. with an estimated incidence of 73,480 cases per annum. Moreover, the hemolytic uremic syndrome (HUS), a sequela of O 157:H7 infection, is the most frequent basis for acute kidney failure in U.S. children. The incidence of non-O157:H7 disease in this country is about half that of O157:H7, or 36,740 cases per year. Worldwide, the single most common serotype of STEC associated with human illness is O157:H7 but other serotypes have also been linked to development of HC and the HUS. The majority of such non-O157 isolates from humans are intimin positive, but intimin-negative strains of STEC have also been incriminated as causes of serious human disease. Because of the potential severity of STEC infection and because O157:H7 has a very low infectious dose 50% and can be spread from person to person, the bacterium is considered a category B biological threat by CDC. The long-term goals of this project are to define at the molecular, cellular, and whole animal levels the pathogenic mechanisms by which STEC cause disease and to develop strategies for prevention and treatment of STEC-mediated HC and HUS. The specific aims are to: 1. assess the cell biology and distribution of the elastase-activatable, highly potent, phage-encoded Stx2d toxin; 2. define the structure function relationship between intimin-gamma of E. coli O157:H7 and the eukaryotic receptor nucleolin and determine whether nucleolin also engages other types of intimin; 3. test the theory that the interaction between intimin-y and nucleolin on the host cell surface activates an intracellular response that is essential for EHEC O157:I-'17 adherence; 4. explore the hypotheses that O157:H7 strain 86-24 can induce intestinal A/E lesions in orally-infected mice if the strain is altered to express Citrobacter rodentium intimin alone or in concert with other C. rodentium LEE-encoded proteins and that such a strain more efficiently delivers Stx2 systemically than does wild-type and is, therefore, more virulent, and; 5. continue to evaluate as vaccine candidates the C-terminal fragments of intimin-gamma from EHEC O 157:H7 and intimin-alpha from enteropathogenic E. coli (EPEC) as well as Stx1 and Stx2 toxoids for capacity to elicit antibodies that inhibit adherence of EHEC O157:H7 and EPEC to tissue culture cells and to neutralize Stx1 and Stx2, respectively, and to assess the protective efficacy of these immunogens alone or in

combination when given to animals either parenterally or orally in transgenic plant cells.

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Grant: 2R01AI020723-19A2

Program Director: RUBIN, FRAN A.

Principal Investigator: SCOTT, JUNE R

PHD

MICROBIOLOGY:BACTERIOLOGY

OGY

Title: Genetic Analysis of S. Pyogenes Virulence Factors

Institution: EMORY UNIVERSITY ATLANTA, GA

Project Period: 1984/06/01-2008/01/31

Streptococcus pyogenes, the group A streptococcus (GAS) is an important human pathogen causing frequent self-limiting diseases which may lead to serious sequelae. In addition, the GAS seems to be "reemerging" as a cause of life-threatening invasive disease. Because of the great diversity of syndromes produced by many strains of GAS, we wish to improve our understanding of the pathogenesis of this organism by focusing on the regulation of expression of its genes, which presumably occurs on interaction with the human host to determine disease outcome. This proposal focuses on the two major global regulators that alter expression of many GAS proteins, including virulence factors. The regulation of expression of Mga (Aim 1) and CovR/S (CsrR/S) (Aim 3) will be studied. The genes controlled by Mga and CovR/S and the mechanisms by which these regulatory proteins control expression of these genes will also be investigated (Aims 2 and 4). We hope these analyses will improve our understanding at the molecular level of the interactions of GAS with its human host and may identify new targets for development of therapies and vaccines.

Grant: 2R01AI021548-19
Program Director: KLEIN, DAVID L
Principal Investigator: BRILES, DAVID E PHD
MICROBIOLOGY:IMMUNOLOGY
Title: Mechanism of Action of Antibody to PspA
Institution: UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL
BIRMINGHAM
Project Period: 1984/08/01-2007/10/31

DESCRIPTION (provided by applicant): Streptococcus pneumoniae is one of the largest causes of death by infectious disease of the elderly worldwide. It is also one of the greatest causes of death among very young children in developing countries. Although the polysaccharide-protein conjugate vaccine is effective at protecting children from bacteremia and sepsis, protection is restricted to the included capsular types. The vaccine is only partially protective against otitis media and its potential for herd immunity is limited. Moreover, the conjugate vaccine is more than 100-fold too expensive for widespread use in the developing world. One way to improve this vaccine, or possibly replace it, would be to use protection-eliciting cross-reactive proteins of pneumococci. Several such proteins have been identified, and one, PspA, has reached clinical trials. PspA is required for full virulence of pneumococci in mice and antibodies to it are protective against sepsis, pneumonia, and carriage. Antibody to PspA can enhance the clearance of pneumococci from the blood of infected animals, and it appears to be able to increase complement deposition on the pneumococcal surface in vitro. Antibodies to PspA promote the attachment of pneumococci to phagocytes, but have not been found to be opsonic (even in the presence of complement) for phagocytosis and killing in vitro. To obtain a better understanding of how antibodies to PspA promote protection in vivo, we will examine several of their known biologic effects in detail. Our investigations will include in vitro conditions that are as close as possible to those in vivo. Investigations of a panel of protective and non-protective monoclonal antibodies, all of which will react with native PspA, will allow us to determine which biologic assays are relevant to in vivo protection. We will also map the epitopes that elicited the monoclonal antibodies. Identification of the in vivo mechanism by which antibody to PspA protects should enable development of a valid surrogate assay for protection, and should improve our understanding of pneumococcal disease. Our analyses of the protection-eliciting and non-protection-eliciting epitopes on PspA should make it possible to design even better PspA vaccines in the future.

Grant: 2R01AI022933-17A2
Program Director: VAN DE VERG, LILLIAN L.
Principal Investigator: HEFFRON, FRED L PHD
Title: Genetic Analysis of Salmonella Virulence
Institution: OREGON HEALTH & SCIENCE UNIVERSITY PORTLAND, OR
Project Period: 1987/04/01-2008/02/29

DESCRIPTION (provided by applicant): Salmonella is a common and important pathogen that kills millions of people each year primarily children in developing countries. It was the first organism to be used as a bioterrorist weapon in the US and is listed as category B in the NIH list of BT agents (*S. enterica* as we describe here). The primary goal of my research is to use Salmonella as a model to understand how intracellular pathogens manipulate host cells to cause disease. Using our expertise with the Salmonella/macrophage model, coupled with genetics, molecular biology and transcriptional profiling - we will further our understanding of how intracellular pathogens regulate virulence gene expression. Our hypothesis is that multiple regulators respond to different cues within cells and that the signal becomes integrated, perhaps by one or a few master regulators, to express specific subsets of virulence factors required for survival and growth within different cells and tissues of the host. Salmonella is a model for studying intracellular pathogenesis without equal because of its established genetics and simple and inexpensive animal model - the mouse. Understanding how Salmonella survives and replicates within the host and how it expresses virulence genes at the appropriate time and place during infection will provide a paradigm for understanding other pathogens.

Grant: 2R01AI023348-14A2
Program Director: KORPELA, JUKKA K.
Principal Investigator: LOW, DAVID A
Title: DNA Methylation and Pili Gene Regulation
Institution: UNIVERSITY OF CALIFORNIA SANTA BARBARA, CA
BARBARA
Project Period: 1987/04/01-2006/10/31

DESCRIPTION (provided by applicant): The goal of this proposal is to understand the mechanisms by which DNA adenine methylase (DAM), leucine-responsive regulatory protein (Lrp), and PapI orchestrate the reversible switch between OFF and ON Pap pili expression states in uropathogenic *Escherichia coli* (UPEC). Since Pap pili are an essential virulence determinant of UPEC, this work has direct application to addressing the problem of urinary tract infections. This work will serve as a paradigm to understand how DNA methylation patterns control heritable gene expression states. The core switch involves PapI-dependent translocation of Lrp between pap promoter proximal sites 1,2,3 and distal sites 4,5,6. The methylation states of two GATC sites within the central pap sites 2 and 5 (GATCprox and GATCdist, respectively) control binding of Lrp and Lrp-PapI. Binding of Lrp to promoter proximal sites represses pap transcription whereas binding of Lrp to distal sites is essential for activation of pap transcription. The first aim is to determine how PapI and DAM control binding of Lrp to sites 1,2,3 and 4,5,6. The hypothesis that PapI enhances binding of Lrp to sites 2 and 5 by interacting with pap DNA sequences and Lrp in a ternary complex will be tested. The base-pair contacts between Lrp-PapI and sites 2 and 5 will be identified by missing contact, SELEX, and methylation analyses. Regulatory mutant pap DNA's which bind Lrp normally but are no longer PapI-responsive will be used to test the hypothesis that methylation of GATCprox facilitates OFF to ON switching by specifically blocking PapI enhancement of Lrp binding at sites 1,2,3. The second aim is to identify amino acids of Lrp that play important roles in responsiveness to PapI and DNA methylation, which will be accomplished by isolation of Lrp mutants with altered responses to these factors and by a genetic suppressor approach using pap mutants isolated in Aim 1. Photocrosslinking studies are proposed to directly identify amino acids within Lrp that interact with sites 2 and 5, and to determine how these interactions are altered by GATC site methylation. Amino acids at the protein-protein binding interface of PapI and Lrp will be identified using yeast one-hybrid and beta-lactamase complementation analyses. The third aim is to analyze real-time in vivo dynamics of phase variation, which will include a test of the hypothesis that DNA replication is required for Pap phase switching. This will be carried out by monitoring Pap pili gene expression by fluorescence activated cell sorting in synchronized cells following induction of Pap I. Further analysis of the methylation states of the pap GATC sites in wild-type and regulator mutant pap operons following passage of the replication fork will be done to link in vitro studies with in vivo switch dynamics. These studies will also provide a detailed framework for understanding epigenetic regulatory mechanisms in other prokaryotes and eukaryotes.

Grant: 2R01AI023988-15
Program Director: PERDUE, SAMUEL S.
Principal Investigator: HOOPER, DAVID C
Title: Quinolone Resistance Mechanisms in *Staphylococcus aureus*
Institution: MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA
Project Period: 1986/09/01-2008/03/31

DESCRIPTION (provided by applicant): Quinolone Resistance Mechanisms in *Staphylococcus aureus*. Quinolone antimicrobials act on two topoisomerases and are used widely in human infections. Their use for common *S. aureus* infections has been limited by emerging resistance. Resistance mechanisms include altered topoisomerases and increased expression of multidrug resistance (MDR) efflux pumps. Defining these mechanisms is key to strategies to avoid resistance. Long-term objectives of the project are to use quinolones and resistant mutants as a model system to study the control and function of topoisomerases and efflux pumps found in many bacteria. Specific aims are (1) to define the roles of novel mutations in topoisomerase IV in effecting resistance and altering enzyme function. Mutant enzymes will be purified and studied for their catalytic functions and binding of DNA and quinolones as well as formation of quinolone-induced DNA cleavage. Aim (2) is to identify the sites of quinolone interaction with complexes of topoisomerase IV, DNA, and quinolone using x-ray crystallography of wildtype and mutant enzymes complexed with DNA and drug. Aim (3) is to identify the mechanism by which the NorR protein regulates expression of the Nora efflux pump and possibly other related pumps using DNA footprinting with *norA* promoter DNA and purified NorR. We will also perform transcriptional profiling of the expression of genes encoding efflux pumps with DNA microarrays hybridized with RNA prepared from strains with mutations in *norR* and *arlS*, both of which affect *norA* expression. Aim (4) is to identify additional factors regulating *norA* expression by purification of a 28-kd protein that in addition to NorR binds upstream of *norA*, identification of the gene encoding, and generating mutants in this gene. Aim (5) is to identify the complement of multidrug resistance pumps and analyze their expression in an abscess model. This work will be done by analysis of genes related to those of known MDR pumps and their regulators and selected cloning and overexpression of these genes in *S. aureus*. Overall patterns of expression of the genes will be compared by transcriptional profiling in DNA microarrays using RNA prepared from bacteria surviving in a subcutaneous abscess in rats and RNA from bacteria grown in vitro.

Grant: 2R01AI024431-18
Program Director: SCHMITT, CLARE K.
Principal Investigator: OBRIG, TOM G PHD
Title: SHIGA TOXIN MODE OF ACTION IN BACTERIAL DISEASE
Institution: UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA
CHARLOTTESVILLE
Project Period: 1990/04/01-2007/12/31

DESCRIPTION (provided by applicant): Shiga toxin-producing E. coli (STEC) is an emerging infectious pathogen that causes in excess of 30,000 cases of disease per year in the United States. STEC, including E. coli O157:H7, is also the leading cause of acute renal failure, hemolytic uremic syndrome (HUS), in young children. No effective preventive modality or therapeutic intervention is currently available for this disease. This project is designed to more fully describe a "window of opportunity" available for treatment and prevention of STEC-associated acute renal failure. In most cases, a three to nine day period of renal inflammation takes place between the appearance of bloody diarrhea and the onset of acute renal failure. It is believed that STEC virulence factors such as Shiga toxin (Stx2) and lipopolysaccharide (LPS) are the primary initiators of the renal disease. These factors elicit production of pro-inflammatory host cytokines and chemokines. This study utilizes a murine model to define the cytokines and chemokines involved and describe how these agents cause migration and accumulation of inflammatory cell types in the kidney. These cell types include neutrophils, monocytes/macrophages and platelets. Mice with mutated cytokine, chemokine, or adherence factor genes are to be employed to determine which of these factors are required in the disease process. In addition, adherence of these cell types to isolated endothelial cells under flow conditions is included to define the inflammatory action of Stx2 and LPS. Studies are also included to show how host cytokines and chemokines further sensitize endothelial cells to Stx2 by activation of intracellular signal transduction pathways. The goal of these studies is to reveal the opportunities available for effective application of therapeutic agents in STEC-associated renal disease.

Grant: 2R01AI024656-14
Program Director: VAN DE VERG, LILLIAN L.
Principal Investigator: MAURELLI, ANTHONY T
Title: Molecular Genetic Analysis of Shigella Pathogenicity
Institution: HENRY M. JACKSON FDN FOR THE ADV MIL/MED ROCKVILLE, MD
Project Period: 1988/12/01-2008/01/31

DESCRIPTION (provided by applicant): Shigella are bacterial pathogens of man which are the causative agents of bacillary dysentery. Over 200 million cases are reported annually and about 650,000 persons die of shigellosis each year. No effective vaccine exists. Recent bioterrorism concerns increase the importance of understanding Shigella pathogenesis since Shigella has been classified as a category B agent on the NIAID's list of priority pathogens for biodefense research. Shigella is a classic example of an invasive, facultative intracellular pathogen that regulates expression of its virulence genes in response to environmental signals. Our long term objectives have focused on two areas of Shigella pathogenesis: regulation of virulence gene expression and molecular characterization of virulence gene function. The specific aims of this proposal are to : 1) examine the mechanisms by which Shigella regulates virulence gene expression after invasion into the host cell cytoplasm; 2) define the genetic and molecular basis of post invasion phenotypes associated with virulence; and 3) elucidate the mechanism by which components of the type III secretion system interact and recognize virulence proteins which are targeted for secretion. Several models and experimental strategies for testing them are proposed. Genetic and biochemical approaches for measuring DNA-protein interactions will be utilized in the first aim. For aim two, cellular microbiology strategies will be combined with bacterial genetics and molecular biology. For aim three, interactions between components of the type III secretion machinery will be detected by suppressor analysis and use of biochemical techniques. In addition, mutant selection will be applied to define secretion signals recognized by this pathway. This research will fill in important gaps in our knowledge of Shigella pathogenesis. An understanding of the mechanisms that control expression of virulence genes after Shigella invasion can lead to the development of specific drugs that block expression of these genes. Information on how Shigella recognize and secrete virulence factors can also reveal novel targets for the design of new therapeutic agents.

Grant: 2R01AI025098-15
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: PERRY, ROBERT D
Title: Characterization of the Hms phenotype of *Yersinia pestis*
Institution: UNIVERSITY OF KENTUCKY LEXINGTON, KY
Project Period: 1993/09/30-2008/01/31

DESCRIPTION (provided by applicant): *Yersinia pestis* primarily causes a zoonotic disease - plague. Although humans are an accidental, dead-end host, bubonic and pneumonic plague has caused widespread loss of human life during recurrent pandemics. Nearly 2,000 human plague cases occur yearly and small epidemics in South America and Southeast Asia are common. In addition, *Y. pestis* has great potential for use as a bioterrorism agent. In nature, the bacterium has an obligate flea-mammal (primarily rodent) life cycle. Survival of plague in nature is dependent upon its ability to propagate in fleas and upon its transmission from fleas to mammals. In this proposal we will focus on determinants of the hemin-storage (Hms) phenotype of *Y. pestis* that are involved in the flea portion of the bacterial life cycle. The Hms⁺ phenotype is required for *Y. pestis* to cause blockage of the proventricular valve of fleas. Blockage of this valve between the esophagus and stomach of the flea is required for transmission of the disease from fleas to mammals. In vitro the Hms⁺ phenotype is characterized by binding of enormous quantities of exogenous hemin or Congo red (CR) dye to the outer membrane fraction of cells grown at 26-34 degrees Celcius but not at higher temperatures. Thus Hms⁺ cells of *Y. pestis* incubated at 26 degrees Celcius on CR agar form red colonies. We now hypothesize that the Hms phenotype of *Y. pestis* is the result of an extracellular matrix or biofilm produced by the Hms system. The mechanisms of proventricular blockage, roles of Hms proteins in biofilm formation, and mechanisms of temperature regulation of the phenotype remain to be elucidated. We propose to continue studies on identifying essential hms genes, analyzing the regulation of expression of the Hms⁺ phenotype, as well as characterizing the biochemical properties and physiological role(s) of the Hms system. The specific aims of this proposal are - 1) genetic analysis of essential hms genes; 2) analysis of the regulation of Hms expression; 3) biochemical analysis of the Hms system; and 4) analysis of the physiological role(s) of the Hms system. These studies will enhance our understanding of bacterial features that are required for *Y. pestis* to block the flea which is essential for the transmission of plague from fleas to mammals. This will facilitate measures to control or eliminate natural reservoirs of plague and control disease transmission. In addition, elucidation of the enzymatic activities of Hms proteins and regulatory mechanisms controlling the synthesis of the extracellular matrix likely produced by the Hms system, may have relevance for biofilm formation by other pathogenic bacteria.

Grant: 2R01AI026195-16
Program Director: VAN DE VERG, LILLIAN L.
Principal Investigator: FALKOW, STANLEY PHD BIOLOGY NEC:BIOL
NEC-UNSPEC
Title: Genetic and Molecular Basis of Bacterial Invasion
Institution: STANFORD UNIVERSITY STANFORD, CA
Project Period: 1988/04/01-2008/03/31

DESCRIPTION (provided by applicant): The thrust of our proposed research is to examine the typhoid model of mouse infection using animals carrying the nramp\resistant allele. In this experimental model of infection, we propose to examine well-characterized bacterial mutants, as well as focusing on the location of the bacteria during the acute phases of infection and during chronic carriage. Because persistent infection is asymptomatic, we rely upon a non-invasive, semi-quantitative photonic detection system to identify infected animals. We will employ bacterial strains carrying fluorescent reporter molecules to follow the location of the bacteria in the tissue, as well as using fluorescence activated sorting (FACS) of infected tissue to locate the host cell populations interacting with the invading bacteria. Using immunohistochemical methods, in-situ hybridization, confocal microscopy with antibody tagged reporter molecules, as well as biochemical assays of tissue, we will determine the precise nature of the host cells persistently infected by Salmonella. We have constructed a spotted DNA microarray of the entire S. typhimurium genome, as well as a 40000-element mouse DNA microarray. We plan to use these experimental tools to monitor both the bacterial response and the host response to infection with particular emphasis on the transition by both the microbe and the host from an acute infectious process to a systemic and eventually persistent infection. Finally, we will employ genetic screening methods based on signature tagged transposon analysis, as well as a new novel DNA microarray-based screen, to identify bacterial genes that are essential for persistent infection and bacterial transmission.

Grant: 2R01AI026289-16
Program Director: HALL, ROBERT H.
Principal Investigator: MEKALANOS, JOHN J PHD
Title: Coordinate Regulation of Bacterial Virulence Factors
Institution: HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA
Project Period: 1988/05/01-2008/04/30

DESCRIPTION (provided by applicant): For the next funding period of AI-26289, our overall goal is to understanding the biology of five pathogenic microorganisms (*Vibrio cholerae*, *Pseudomonas aeruginosa*, *Helicobacter pylori*, *Haemophilus influenzae*, and *Escherichia coli*) in order to discover anti-infective strategies based on the use of small molecule inhibitors. These studies will be horizontally integrated by use of common technologies and genetic approaches. For example, we will use differential fluorescence induction (DFI) to define and study genes expressed in vivo or associated with virulence regulons of biological interest. Genomic microarrays will be used to measure gene expression at the global transcriptional level under various growth conditions, in various defined mutants, and under a series of imposed stresses. These stress challenges will include exposure to host milieu during infection, exposure to host cells, withdrawal of essential gene products through conditional expression, and exposure to toxic compounds, toxic protein aptamers, and inhibitory antibiotics. We will use a variety of methods including the new DNA-chip based "TraSH method" to identify genes required for bacterial growth and viability in vitro and in vivo. We will attempt to identify the function of new essential proteins by characterizing complexes they form with other proteins through micro liquid chromatography tandem mass spectrometry (microLCMS) analysis. Other proteomic projects will include the use of microLCMS to define proteins expressed on the surface of bacteria grown in vitro and in vivo. We will also explore the use of "protein chips" as a means of doing protein-protein interaction analysis and as a new tool for studying host immune responses. Computational methods will be used to mine expression and genomic databases for interesting potential virulence or essential gene products, which will then be analyzed genetically for attenuation in experimental animals. We will use the information we gain on essential processes in pathogenic bacteria to devise sensitive bioscreens that can detect small molecule inhibitors of these processes. Finally, we will screen diverse combinatorial compound libraries in high-throughput formats for "hits" that pharmacologically interfere with essential and virulence related functions.

Grant: 2R01AI026328-12A2
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: KELLY, KATHLEEN A PHD
Title: T-Cell Mediated Immunity in Chlamydial Genital Infection
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 1988/04/01-2007/11/30

DESCRIPTION (provided by applicant): Chlamydia trachomatis infection remains a major cause of pelvic inflammatory disease, and often leads to fallopian tube injury and infertility in humans. While effective antibiotics are available, asymptomatic infection may ascend to the upper genital tract (GT) and cause irreversible tissue damage before it is discovered. One strategy for avoiding injury to the upper GT is through preventative vaccination. However, the anti-chlamydial CD4 T-helper type 1 (Th1) response that develops to eradicate infection is also thought to participate in upper GT injury. The goal of this project is to identify mechanisms that regulate chlamydial immunity in the upper GT to better understand the basis of upper GT injury. We recently reported that CD4 cells are primarily recruited to the upper but not the lower GT and that Th1 attractant chemokines are produced primarily in the upper GT during infection. Based on those findings we hypothesize that the ability to recruit T cells differs between the upper and lower GT. We have also found that Th1 cells are not the only subset recruited to the GT. Anti-Chlamydia-specific T cells that produce IL-10 are also recruited to the GT during infection. T cells that secrete IL-10 may interfere with a Th1 response and enhance upper GT injury. Therefore, it will be important in validating the hypothesis to determine whether the IL-10 producing T cells that are recruited to the GT can modulate the Th1 response against Chlamydia. To test this hypothesis we will 1) Identify mechanism(s) that control the enhanced recruitment of Th1 CD4 cells to the upper GT, 2) Determine if altering Th1 CD4 cell recruitment improves immunity against Chlamydia infection. 3) Determine if IL-10 producing T cell subsets (Th2, Tr1) impede anti-chlamydial Th1 immunity. To achieve these goals, we will focus on identifying chemokine and chemokine receptor ligands that mediate Th1 cell recruitment to the GT. In addition, we will attempt to boost immunity in the lower GT by the delivery of these Th1-attractant chemokines to the vaginal region using adenoviruses that secrete Th1-attractant chemokines. Also, using IL-10 knockout and transgenic mice we will determine whether IL-10 producing T cell subsets influence anti-Chlamydia immunity and the development of upper GT injury.

Grant: 2R01AI027655-16
Program Director: HALL, ROBERT H.
Principal Investigator: PORTNOY, DANIEL A PHD
Title: Listeria Hemolysis and Escape From a Vacuole
Institution: UNIVERSITY OF CALIFORNIA BERKELEY BERKELEY, CA
Project Period: 1988/06/15-2008/05/31

DESCRIPTION (provided by applicant): *Listeria monocytogenes* is a highly tractable intracellular pathogen and a cause of serious food-borne illness in humans. A primary determinant of *L. monocytogenes* pathogenesis is a secreted pore-forming protein referred to as listeriolysin O (LLO). LLO mediates escape of *L. monocytogenes* from a phagocytic vacuole and is absolutely essential for virulence. LLO has a number of unique properties that prevent its activity in the host cytosol including an acidic pH optimum and a PEST-like sequence. The goals of the current proposal are to identify both bacterial and host components that control LLO compartmentalization, and to determine why mutants that fail to properly compartmentalize LLO activity are avirulent. In Aim I, two genetic selection screens are proposed to identify bacterial proteins that control LLO secretion in a vacuole, and two other screens are proposed to identify bacterial proteins that prevent LLO secretion in the cytosol. The intracellular phenotypes of the mutants will be characterized in a variety of tissue culture assays, and secretion defects examined biochemically. The role of two recently identified autolysins whose synthesis or activation appears to be vacuole-specific will be examined. In Aim II, it will be determined why cytotoxic mutants are less virulent. One hypothesis that will be directly tested is that cytotoxic mutants become extracellular and are targeted by neutrophils. The role of neutrophils and neutrophil chemotaxis will be addressed in neutropenic and knockout mice. In Aim III, a functional genomics approach will be used to identify host proteins that control escape of *L. monocytogenes* from a vacuole and that prevent LLO toxicity in the host cytosol. The host cells to be used in these studies are a phagocytic *Drosophila* cell line that is extremely sensitive to double-stranded RNA interference (RNAi). RNAi specific for 7800 *Drosophila* genes will be produced and used to screen, microscopically, for host genes that encode proteins controlling LLO compartmentalization.

Grant: 2R01AI028797-10A1
Program Director: KORPELA, JUKKA K.
Principal Investigator: DZIARSKI, ROMAN
Title: Mechanism of pathogenic activity of peptidoglycan
Institution: INDIANA UNIV-PURDUE UNIV AT INDIANAPOLIS, IN
INDIANAPOLIS
Project Period: 1992/07/01-2007/11/30

DESCRIPTION (provided by applicant): Bacterial infections are still the major cause of morbidity and mortality, and innate immunity is the first line of host defense against infections. The overall objective of this project is to elucidate how innate immunity protects the host from infections with Gram-positive bacteria and how the host response to bacterial components causes clinical manifestations and pathologic changes associated with infections. This project investigates the main component of Gram-positive bacterial cell walls, peptidoglycan, which can reproduce several major clinical manifestations of bacterial infections. This laboratory has identified a novel family of four pattern recognition proteins in humans that recognize peptidoglycan (PGRPs). These human PGRPs are differentially expressed in the bone marrow, neutrophils, liver, esophagus, tonsils, and thymus, and are highly homologous to the family of 12 insect PGRPs. This project will test the hypothesis that mammalian PGRPs interact with peptidoglycan and bacteria and are involved in innate immunity to bacteria. Specifically, the objective will be to identify the function of PGRPs in mammalian immunity using two approaches: First, it will be determined if PGRP-S knockout mice (generated in this laboratory) are immunodeficient, by testing the following aspects of their immunity: (i) development of lymphoid organs and cells; (ii) susceptibility to infections; (iii) inflammatory responses; (iv) induction of cytokines and chemokines; (v) chemotactic and phagocytic responses; and (vi) antibody responses. Second, the functions of the remaining PGRPs (PGRP-L, PGRP-I-alpha, and PGRP-I-beta) will be determined by identifying: (i) tissues and cell types that express these PGRPs; (ii) subcellular localization of each PGRP; (iii) their ligands; (iv) association of PGRPs with other proteins; (v) ability of these PGRPs to induce acute-phase response and uptake of bacteria; (vi) functional domains of these PGRPs; and (vii) inhibitory effects of soluble PGRP-S on the functions of transmembrane PGRPs. This study may discover a new mechanism of innate immunity and a new immunodeficiency, and may provide tools for developing new preventive and therapeutic measures against infections with Gram-positive bacteria.

Grant: 2R01AI029092-12A1
Program Director: AULTMAN, KATHRYN S.
Principal Investigator: DEAN, DONALD H PHD
Title: Functional Domains of *Bacillus thuringiensis* Endotoxins
Institution: OHIO STATE UNIVERSITY COLUMBUS, OH
Project Period: 1989/07/01-2008/02/29

DESCRIPTION (provided by the applicant): *Bacillus thuringiensis* is a microbial insecticide that is widely used to control insects, including mosquitoes and black flies. The long-range goal of this project is to investigate the binding and mechanism of action of several mosquitocidal proteins against key pestiferous mosquito species, *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus*. The main mosquitocidal toxins of interest are the toxins of *B. thuringiensis* var. *israelensis* (Bti), Cry4Aa, Cry4Ab and Cry11Aa. Other mosquitocidal toxins, Cry11Ba, Cry19Aa and Cry2Aa, will also be investigated. The hypothesis to be tested is that these toxins bind to specific receptors on the mosquito midgut as recognized in model insect-toxin studies (the Lepidoptera-toxin paradigm); i.e., an array of mosquito midgut proteins (cadherin-like and aminopeptidases) and glycoproteins bind the toxins; and, that domains II and III of the are the interacting binding epitopes. A corollary hypothesis is that the reduced ability of mosquitoes to develop resistance to Bti is due to a combination of toxins (Cry4Aa, Cry4Ab and Cry11Aa) each of which binds to a unique receptor or non-competing binding site. The specific aims of the proposal are: (1) Test the competition, saturation and irreversible binding of Cry4Aa, 4Ba, 11Aa, 11Ba, 19Aa and 2Aa toxins to mosquito BBMV and purified receptors. New mosquitocidal activity has been introduced into Cry4Ba (*Culex* activity) and 19Aa (*Aedes* activity). The mechanistic basis for these new activities will be investigated. (2) Examine the mechanism of action of mosquitocidal of these toxins, in comparison to the Lepidoptera toxins paradigm; specifically, to define the binding epitopes of these toxins to brush border membrane vesicles (BBMV) of the mosquitoes *Aedes aegypti*, *Anopheles gambiae* and *Culex quinquefasciatus*. (3) Examine the nature of mosquito midgut receptors in relation to what has been learned from Lepidoptera receptor paradigm; specifically, to identify the specific binding of these mosquitocidal Cry proteins on aminopeptidases, cadherin-like proteins, glycoproteins and other potential receptors of these mosquitoes.

Grant: 2R01AI029743-10
Program Director: BAKER, PHILLIP J.
Principal Investigator: CHARON, NYLES W PHD
Title: An analysis of *Borrelia burgdorferi* motility
Institution: WEST VIRGINIA UNIVERSITY MORGANTOWN, WV
Project Period: 1990/06/01-2008/02/28

DESCRIPTION (provided by applicant): Lyme disease is the most prevalent arthropod borne infection in the United States, and has shown a steady increase in incidence since its discovery. The spirochete *Borrelia burgdorferi* is the causative agent. Very little is known about the *B. burgdorferi* virulence attributes that bring disease to the host. The present proposal focuses on the chemotaxis and motility of *B. burgdorferi*, and their role in the disease process. Chemotaxis and motility are likely to be important virulence attributes, as these organisms penetrate into tissues and organs where other bacteria fail to invade. Basic to an understanding of chemotaxis on a molecular level, in Aim 1 we will identify those compounds that serve as attractants. Putative chemoattractants will be identified from information drawn from the genome sequence and known metabolism of *B. burgdorferi*. The chemotactic ability of these compounds will be tested using established methodology. Both high passage (avirulent) and low passage (virulent) strains will be tested under appropriate environmental conditions. In Aim 2, we propose to construct both insertion-deletion and in-frame deletions mutations in specific motility and chemotaxis genes. Both high and low passage strains will serve as parental strains. We hypothesize that each of these mutants will have a distinct phenotype. These phenotypes will be analyzed with respect to motility, chemotaxis, structure, western blot, proteomics and transcriptional analysis using DNA-microarray technology. To insure that a given phenotype is due to the targeted mutation being studied, we will use gene-complementation analysis. In Aim 3, we will test the hypothesis that motility and chemotaxis are important virulence attributes of *B. burgdorferi*. To test this hypothesis, we will use standard cell penetration assays, and the mouse model of Lyme disease. We predict that the motility and chemotaxis mutants will be less invasive and virulent than the parental strains in both of these assays. The experiments proposed should yield information with respect to the roles of chemotaxis and motility of *B. burgdorferi* in Lyme disease, and perhaps lay the foundation for new methods for prevention and treatment.

Grant: 2R01AI031254-10A1
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: GIBSON, BRADFORD W PHD
Title: Structure and Function of Haemophilus ducreyi LOS
Institution: BUCK INSTITUTE FOR AGE RESEARCH NOVATO, CA
Project Period: 1994/09/01-2008/03/31

DESCRIPTION (provided by applicant): Haemophilus ducreyi is a gram-negative human pathogen and causes the genital ulcer disease 'chancroid'. Although uncommon in the U.S, outbreaks continue in some regional areas and chancroid remains a significant risk factor for the transmission of HIV in developing countries. Moreover, the emergence of antibiotic resistant strains suggest that a molecular-based understanding of the events that enable this human pathogen to infect, survive, form lesions, and disseminate is needed if we are to contain or eliminate this disease worldwide. Outer-membrane lipooligosaccharides (LOS) have been identified as a major virulence factor in several mucosal pathogens. LOS has been implicated in the adherence and invasion of human tissues, serum resistance, and resistance to phagocytosis and complement mediated killing. The previous application was concerned with elucidating the structures of these complex glycolipids and assessing biological activities. Ultimately, our goal is to provide the molecular underpinnings for the development of new carbohydrate-based vaccines and drugs targeting critical host-pathogen interactions. To do this, we need to understand the repertoire of LOS-glycoforms expressed by this pathogen, the function(s) of these glycoforms, and how LOS interacts with host proteins. In this renewal, we propose to explore the pathway that leads to the expression of LOS terminating in sialic acid and its acceptor, N-acetyllactosamine, using chemistry and structural biology tools. Sialic acid is used by many human pathogens, including bacteria, viruses and trypanosomes, and is an important sugar in cell recognition mechanisms throughout biology. Given the relative simplicity of the disease and the well-defined structures for H. ducreyi LOS, this organism represents an ideal model system for studying the pathway(s) leading to sialic acid expression in these surface glycolipids and the role of sialylated LOS in host-pathogen interactions. The hypothesis we wish to examine in this proposal is that bacterial surface LOS containing sialic acid play important roles in the infection process and are key to reaching an understanding of how carbohydrates and sialic acid mediate host-pathogen interactions. To address this hypothesis, three specific aims will be explored as follows: Aim #1, investigate the sialic acid pathway leading to the expression of outer-membrane sialylated LOS glycoforms; Aim #2, examine the relationships among the polylactosamine, sialyllactosamine and asialo- oligosaccharide branch pathways in LOS biosynthesis; Aim #3, develop and employ sialic acid analogs as inhibitor of the sialic acid pathway in H. ducreyi.

Grant: 2R01AI032248-11A1
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: DE LA MAZA, LUIS M MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC
Title: Immunopathology of Tubal Infertility
Institution: UNIVERSITY OF CALIFORNIA IRVINE IRVINE, CA
Project Period: 2003/09/30-2008/01/31

DESCRIPTION (provided by applicant): Chlamydia trachomatis is one of the most common pathogens involved in sexually transmitted diseases. In most instances, particularly in women, the infection is asymptomatic and thus, therapeutic measures cannot be initiated. Even in symptomatic cases, unless adequate therapy is implemented in a timely fashion, the patient may end up suffering from long term sequelae including chronic abdominal pain, ectopic pregnancy and infertility. In this proposal we want to test the hypothesis that a vaccine consisting of the C. trachomatis major outer membrane protein (MOMP) will be able to induce protection in mice against a genital challenge with the C. trachomatis mouse pneumonitis (MoPn) biovar. To achieve this goal we want to utilize a MOMP preparation extracted from native organisms that following purification, has been refolded. Adjuvants, that can be utilized in humans, including CpG, ISCOM, Montanide and DNA plasmids will be tested in mice for their ability to enhance the immunogenicity of the MOMP. In addition, in an effort to optimize a protective immune response, we will test different routes of vaccination. In the immunized animals we will be assessing the parameters that are critical for protection using different approaches. We will first compare the immune response in protected and control groups of three different strains of mice, and will attempt to identify epitopes of the MOMP recognized by B and T cells. Another group of immunocompetent animals will be first immunized with MOMP and subsequently, will be treated with antibodies to block CD4+ and CD8+ T cells and B cells before they are challenged. In addition, we will transfer CD4+ and CD8+ T cells and B cells and antibodies from immunized mice to naive animals before they are challenged. Also, we will use anti-ML-12 and anti-IL-4 antibodies to characterize the role that Th1 and Th2 cells have in protection. Furthermore, to identify the cytokines involved in the eradication of Chlamydia, MOMP-immunized mice will be treated with anti-IFN-g and anti-TNF-a antibodies before they are challenged. In conclusion, our goals are to establish an immunization protocol, utilizing a purified and folded MOMP preparation, that can protect mice against a genital challenge, and to characterize the immune components induced by the folded MOMP that are critical for protection.

Grant: 2R01AI032947-11
Program Director: BAKER, PHILLIP J.
Principal Investigator: FIKRIG, EROL MD MEDICINE
Title: BORRELIA BURGDORFERI INTERACTIONS WITH IXODES SCAPULARIS
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 1994/05/01-2008/04/30

DESCRIPTION (provided by the applicant): This proposal will investigate the interactions between *Borrelia burgdorferi* and *Ixodes scapularis* ticks, in order to better understand Lyme disease pathogenesis. We will explore the hypothesis that the specific association between *B. burgdorferi* outer surface protein (Osp) A and the Tick Receptor for OspA (TROSPA) is required for the successful colonization of *I. scapularis* by the spirochete. We will focus on examining how OspA interacts with the arthropod vector because our published and preliminary studies demonstrate that OspA facilitates the adherence of *B. burgdorferi* to the tick gut. The importance of OspA in the attachment of *B. burgdorferi* to the *I. scapularis* gut will be carefully examined. We will then identify, clone and characterize the TROSPA gene, express and purify TROSPA in recombinant form, and examine the interactions between OspA and TROSPA. In vitro and in vivo studies will then determine whether antibodies to TROSPA, or OspA peptides, can interfere with spirochete colonization of *I. scapularis*. Finally, we will assess the influence of *B. burgdorferi* on the expression of the TROSPA gene, exploring the hypothesis that *B. burgdorferi* upregulate TROSPA within the vector, in part to facilitate survival of the spirochete. The efforts on OspA and TROSPA will be the main focus of the grant proposal. It is also possible; however, that OspA is not the only *B. burgdorferi* ligand that interacts with *I. scapularis*, and we may consider other *B. burgdorferi* genes that are expressed within *I. scapularis* and that may associate with the vector as our research progresses. These studies will explore the intimate relationship between *B. burgdorferi* and *I. scapularis* - with an emphasis on OspA and TROSPA - leading to a greater appreciation of the *B. burgdorferi* lifecycle and new approaches to interfere with spirochete transmission.

Grant: 2R01AI032983-07A2

Program Director: CHALLBERG, MARK D.

Principal Investigator: LYLES, DOUGLAS S PHD BIOCHEMISTRY:FAT
AND LIPID

Title: Cellular interactions of viral matrix protein

Institution: WAKE FOREST UNIVERSITY HEALTH WINSTON-SALEM, NC
SCIENCES

Project Period: 1994/05/01-2008/01/31

DESCRIPTION (provided by applicant): Many viruses inhibit the expression of host genes. In most cases the role of the virus-induced inhibition of host gene expression is to inhibit the host antiviral response, particularly the synthesis of type I (alpha/beta) interferons. An important consequence of the inhibition of host gene expression is that it may promote programmed cell death or apoptosis in host cells. The proposed experiments address the mechanisms of inhibition of host gene expression and the consequences of this inhibition for viral induction of an interferon response or induction of apoptosis using the prototype rhabdovirus, vesicular stomatitis virus (VSV). We have established that the viral matrix (M) protein plays a major role in the inhibition of host gene expression by VSV. Aim 1 is to determine the mechanism of inhibition of host transcription by M protein. We have shown that inhibition of host RNA polymerase II-dependent transcription is due to inactivation of the basal transcription factor TFIID. We will determine changes that have occurred in TFIID from infected cells that account for the inhibition using immuno-affinity purified, epitope-tagged TFIID. Aim 2 is to determine the mechanism by which M protein regulates type I interferon gene expression in host cells. We will determine the extent to which M protein inhibits interferon gene expression by inhibition of basal transcription factors, upstream activators of interferon gene expression, or nuclear-cytoplasmic transport of interferon mRNA. Aim 3 is to determine the mechanism by which M protein regulates induction of apoptosis in host cells. We have established that M protein is a potent inducer of apoptosis when expressed in the absence of other viral components. However, there are at least two VSV components that contribute to induction of apoptosis in the context of a virus infection. We will analyze the molecular components involved in activation of apoptosis by M protein and the other viral component. Infectious VSV cDNA clones will be used to identify other viral components involved in the induction of apoptosis by mapping the sequences of previously isolated viral mutants that enhance their ability to induce apoptosis. These experiments will provide a clearer understanding of the molecular basis for viral pathogenesis including the mechanism for the inhibition of host transcription, the inhibition of interferon production, and the activation of apoptosis.

Grant: 2R01AI033481-10
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: PERRY, ROBERT D
Title: Iron Transport and Regulation in *Yersinia Pestis*
Institution: UNIVERSITY OF KENTUCKY LEXINGTON, KY
Project Period: 1993/07/01-2008/03/31

DESCRIPTION (provided by applicant): For over two thousand years *Yersinia pestis*, the causative agent of bubonic and pneumonic plague, has caused widespread loss of human life during recurrent pandemics. Modern, more contained epidemics are common in South America and Madagascar. In addition, *Y. pestis* is a category A bioterrorism agent with natural zoonotic foci as readily available sources of the organism on nearly every continent. The ability of pathogens to acquire iron from their hosts is one critical parameter in the outcome of the infectious process. *Y. pestis* encodes nine potential inorganic iron transport systems and two heme/hemoprotein transport systems. Of these, the siderophore-dependent yersiniabactin (Ybt) iron transport system and the Yfe ABC transporter are the most important systems for acquisition of inorganic iron. Ybt is essential in the early stages of bubonic plague and mutations in this system are avirulent in mice infected subcutaneously (SC). However, Ybt- mutants are fully virulent via an intravenous (IV) route of infection. Yfe plays an important role during the later stages of the infection - a Ybt-Yfe- mutant is completely avirulent in mice by IV injection. By SC injection, a Yfe- mutant is 75-fold less virulent than its Yfe+ parent suggesting that the Ybt system can partially compensate for a lack of the Yfe system but it is clearly not as effective in the later stages of disease. Expression of both systems is repressed by iron through the iron-responsive regulatory protein Fur. In addition, the Ybt system is activated by an AraC-type regulator, YbtA, possibly acting in concert with the Ybt siderophore. The Yfe system is also repressed by excess manganese through the Fur protein. In vivo repression by manganese and Fur is a unique regulatory mechanism. The specific aims of this proposal are to continue characterizing genetic and biochemical aspects of 1) the Ybt and 2) the Yfe transport systems and 3) to analyze the expression of these two systems in vivo and determine their roles in systemic spread of the disease. We will identify any remaining elements necessary for the function of these systems, examine the regulatory components controlling expression of these systems in vitro and in vivo, and determine the role of these systems in the infectious disease process of plague. An understanding of the components and functions of these two-transport system may lead to their use as protective antigens or as targets for new drugs. Our studies will also provide insights into the role of Ybt and Yfe in the pathogenesis of plague and other disease-causing organisms and into the general importance of iron acquisition in bacterial disease processes.

Grant: 2R01AI033517-10
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: STEPHENS, DAVID S
Title: Meningococcal Lipooligosaccharide & Mucosal Pathogenesis
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 1993/01/01-2007/12/31

DESCRIPTION (provided by applicant): The long-term objectives of this research project are to help define the molecular basis of meningococcal endotoxin (lipooligosaccharide [LOS]), a critical virulence factor of the human pathogen *Neisseria meningitidis*. Meningococcal infections remain devastating in the United States and worldwide. Current meningococcal vaccines have major limitations and antibiotic-resistant meningococci are emerging, emphasizing the need to better understand the molecular mechanisms involved in meningococcal pathogenesis. This application is directed at understanding the structural-functional relationships and heterogeneity of the meningococcal LOS inner core, Hep/2[GlcNAc/(0/1), Glc/(0/1)] PEA/(0/1/2)-KDO/2 Lipid A. LOS inner core structure influences LOS assembly, human immune recognition of the meningococcus and the interactions of LOS with soluble and cellular human proteins. The goals are to understand meningococcal LOS inner core biosynthesis and how meningococcal LOS inner core structure activates TLR4 and influences recognition by human antibody. In Specific Aim 1, the molecular basis of the meningococcal LOS inner core assembly and heterogeneity will be further characterized by: (a) structural and genetic analysis of defined mutants that express only lipid A; (b) identification and characterization of meningococcal genes necessary for the variable phosphorylation of meningococcal lipid A; (c) identification and characterization of meningococcal genes involved in the addition and/or removal of phosphate of Hep II. In Specific Aim 2, the importance of meningococcal LOS inner core structure and heterogeneity in: (a) activation of human Toll 4 receptor (TLR4), and (b) recognition of meningococci by human antibodies will be determined. To accomplish these Aims, genetically defined LOS mutants of meningococci; analytical biochemistry and purified LOS structures; and established human cell and tissue assays will be utilized. Data obtained from the proposed studies has application to the design of meningococcal vaccines currently in development (e.g., inclusion of truncated LOS molecules in outer membrane vesicle or conjugate vaccines) and to the development of therapeutic agents that may treat meningococcal sepsis and meningitis. The studies will also continue to define differences between enteric endotoxin and the unique structure and biology of meningococcal endotoxin.

Grant: 2R01AI034428-07A2
Program Director: VAN DE VERG, LILLIAN L.
Principal Investigator: PICKING, WILLIAM D PHD
Title: Structure and Function of IpaC from *Shigella flexneri*
Institution: UNIVERSITY OF KANSAS LAWRENCE LAWRENCE, KS
Project Period: 1997/09/01-2007/01/31

DESCRIPTION (provided by applicant): *Shigella flexneri* is a gram-negative enteric pathogen that causes bacillary dysentery (shigellosis), which continues to be an important worldwide public health problem. An essential step in the pathogenesis of shigellosis is bacterial invasion of the epithelial cells of the colon. Invasion plasmid antigen C (IpaC) is the effector protein that subverts normal epithelial cell signaling to promote *Shigella* uptake. Our lab has contributed a number of advances in understanding the structure-function relationship of IpaC; however, there has been little focus thus far on determining the biochemistry of IpaC-mediated actin polymerization during epithelial cell invasion. This is partly due to difficulties in purifying and handling this protein. The long-range goal of our laboratory is to determine the precise functional and structural organization of IpaC and to elucidate the molecular mechanism of and the structural basis for IpaC interaction with and subversion of the host cell cytoskeleton. The specific aims of this project are: 1) to determine the outcome of IpaC's association with the host GTPase Cdc42; 2) to determine the IpaC features responsible for direct nucleation of host cell actin which may contribute to efficient *Shigella* entry into epithelial cells; and 3) to identify the sequences and structures located at the IpaC C-terminus that are responsible for its effector activity and its ability to promote vacuolar escape. A common theme in the pathogenesis of many gram-negative bacteria is the specific delivery of effector proteins to eukaryotic cells to subvert the normal target cell signaling mechanisms. For *S. flexneri*, the outcome of this cross talk is host cytoskeletal rearrangement and subsequent bacterial entry. IpaC carries out this interspecies cellular communication by a mechanism that is quite unique relative to those identified in other gram-negative bacterial pathogens. Completion of this work will reveal important new principles of the mechanism by which bacteria trigger epithelial cells to become "phagocytic" for the benefit of the pathogen. This will help in identifying targets for chemotherapeutic control of infection by *Shigella* and related human pathogens. Moreover, novel bacterial proteins capable of modulating eukaryotic pathways are often innovative and valuable tools for future work in cell biology. The novel nature of IpaC's signaling mechanisms provide promise that it, too, represents a new and useful cell biology tool.

Grant: 2R01AI034431-06A2
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: HAAKE, DAVID A
Title: Outer Membrane Proteins of Pathogenic Leptospira Species
Institution: BRENTWOOD BIOMEDICAL RESEARCH LOS ANGELES, CA
INSTITUTE
Project Period: 1996/05/01-2006/12/31

DESCRIPTION (provided by applicant): Leptospirosis is a common zoonotic disease, typically transmitted to humans by rat exposure. Leptospirosis is emerging in areas of the world undergoing rapid urbanization and was recently designated by the National Institutes of Health as an Emerging Infectious Disease. Research is urgently needed to understand how these pathogenic spirochetes interact with their mammalian hosts. The focus of this proposal is to elucidate the molecular mechanisms of pathogenesis and immunity mediated by outer membrane proteins (OMPs). Our laboratory, a leader in this field, has developed strategies for membrane fractionation and has described genes encoding six leptospiral OMPs. We have shown that two of these OMPs, a porin (OmpL1) and a surface-exposed lipoprotein (LipL41) are synergistically immunoprotective in the hamster model of leptospirosis. Recently, we have begun to study the function of leptospiral OMPs using genetic manipulation of leptospire. In the Progress Report we announce the discovery of a novel class of leptospiral proteins containing Bacterial ImmunoGlobulin-like (Big) repeats. The leptospiral Big proteins are structurally related to the Intimin/Invasin family of bacterial adhesins. One of these leptospiral Big proteins, designated BigL3, confers a cellular adhesin phenotype on E. coli. Specific Aim 1 describes studies designed to localize BigL3 and other novel candidate OMPs to be identified through emerging genomic sequence and proteomics data. The goal of Specific Aim 2 is to examine the role of OMPs in mechanisms of pathogenesis and immunity using in vitro assays of adhesion, bactericidal activity, growth inhibition, and opsonophagocytosis. Specific Aim 3 will determine whether any of the OMPs found to play a role in mechanisms of pathogenesis and immunity in Specific Aim 2 are immunoprotective in mucosal, subcutaneous, and intraperitoneal challenge models of hamster leptospirosis. In Specific Aim 4, epitope mapping and monoclonal antibody studies will be performed with the goal of identifying biologically relevant OMP epitopes. The information gained from these studies will be essential for development of rational approaches for the diagnosis, prevention, and treatment of leptospirosis.

Grant: 2R01AI034501-11
Program Director: HALL, ROBERT H.
Principal Investigator: HOL, WIM G PHD
Title: Crystallography of Cholera and Heat Labile Enterotoxins
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 1993/09/01-2008/02/29

DESCRIPTION (provided by applicant): The proposed project uses crystallographic studies to unravel the interactions of two bacterial toxins, cholera toxin (CT) and Escherichia coli heat-labile enterotoxin (LT), with a range of natural substrates and interacting proteins, as well as with designed ligands and inhibitors. The severe infectious diarrhea caused by CT may result in death within a few hours, while the milder diarrhea caused by enterotoxigenic LT-producing E. coli is mainly a traveler's discomfort for inhabitants of industrialized countries, but a major cause of infant death in the third world. CT and LT are remarkably sophisticated hetero-hexameric AB₅ toxins that have many intriguing yet unresolved properties concerning their entry into the cytoplasm of the host cell, their catalytic mechanism, and the mode of secretion of CT from Vibrio cholerae. Based on significant results in the previous grant period we propose in the coming period to tackle, in collaboration with several groups with complementary expertise, structural studies to elucidate the following regarding CT and LT: 1. the mode of binding the substrates NAD and arginine-containing peptides, and the enzymatic mechanism of the ADP-ribosylating A-subunit, based on our recent success in determining the three-dimensional structure of a constitutively active form of CT. This will include a just initiated structure-based drug design collaborative project employing novel guanidine-based chemistry aimed at blocking the A-subunit; 2. the interactions of CT and LT with host proteins such as ADP-Ribosylation Factors (ARFs); 3. the design of ultra-high affinity GM1 receptor antagonists of the B-pentamer by employing multi-ring system-containing monovalent ligands and new "linkers" to obtain a next generation of our successful modular pentavalent and decavalent macroligands; 4. the structure of EpsC, a periplasmic inner membrane-anchored protein from the extracellular protein secretion apparatus in V. cholerae, and its interactions with CT; 5. the structure of the multimeric pore protein EpsD in the outer membrane of V. cholerae and its interactions with EpsC, and possibly, with cholera toxin.

Grant: 2R01AI034503-09
Program Director: RUBIN, FRAN A.
Principal Investigator: CLEARY, PAUL P
Title: Intracellular Infection by Invasive Group A Streptococci
Institution: UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN
Project Period: 1994/12/01-2006/11/30

DESCRIPTION (provided by applicant): This project will define the relationship between intracellular invasion and group A streptococcal pathogenesis. Experiments will further investigate the molecular mechanisms by which streptococci commandeer the extracellular matrix (ECM) and receptor signaling systems of host cells to reach an intracellular state. In vivo experiments will investigate the role of intracellular invasion in persistent infection of nasal associated lymphoid tissue, employing a murine intranasal infection model. Experiments will evaluate whether streptococci preferentially infect this tissue. We propose that persistence depends on the potential of streptococci to be efficiently ingested by specific cells in this lymphoid tissue. Information gained from this study may identify new targets for antibiotic development, and increase our understanding of the mechanisms by which this pathogen persists in human populations.

Grant: 2R01AI034829-14
Program Director: VAN DE VERG, LILLIAN L.
Principal Investigator: MCCLELLAND, MICHAEL PHD MOLECULAR GENETICS
Title: Comparative Genome Analysis of Salmonella Species
Institution: SIDNEY KIMMEL CANCER CENTER SAN DIEGO, CA
Project Period: 1989/12/15-2007/02/28

DESCRIPTION (provided by applicant): There are over 2,000 known serovars of *Salmonella enterica*. These serovars differ dramatically in their host range and pathogenicity. Humans suffer tens of millions of infections and perhaps hundreds of thousands of deaths each year from infection by *Salmonella enterica*. About 100 serovars in subspecies I are responsible for more than 99 percent of these infections. The *Salmonella* genome sequences obtained, so far, indicate that serovars differ in the presence or absence of many hundreds of genes and these differences are presumably responsible for some of the differences in host range and pathogenicity. This project will characterize many of the gene differences among unsequenced serovars that are major pathogens of humans and domestic animals. In the previous grant period we placed on a microarray PCR products for almost all of the genes identified, so far, in the sequenced *Salmonella*. Aim 1: New *Salmonella enterica* genes will be placed on the array as they become available. Aim 2: The array will be used to probe unsequenced *Salmonella enterica* subspecies I genomes to determine the distribution of gene homologues among serovars. Aim 3: Sequenced enterobacterial genomes reveal "hotspots" of diversity at certain locations where insertion, deletion or replacement events, involving one or more genes, distinguish genomes from each other. These differences will be characterized in unsequenced genomes by PCR and also by direct genome sequencing, capturing some of the variation not found in the currently sequenced genomes. Aim 4: The consequences of insertion/deletion and rearrangement on the otherwise highly conserved order of the *Salmonella* genome will be studied. The information gained in these four aims will be a step forward in understanding some of the evolutionary mechanisms behind the phenomenal diversity in host-range and pathogenicity of the *Salmonella*. This information should also be useful in developing tools for epidemiology and could lead to revisions of *Salmonella* taxonomy, which is currently largely reliant on phage typing and immunological measurements of surface antigens. As a member of *S. enterica* subspecies 1 was the only bacteria previously used for a bioterror attack in the USA, prior to the use of Anthrax, such studies take on an added dimension.

Grant: 2R01AI035257-11
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: DRLICA, KARL PHD CELL BIOL
NEC:MOLECULAR BIOLOG
Title: DNA gyrase and quinolone resistance in tuberculosis
Institution: PUBLIC HEALTH RESEARCH INSTITUTE NEWARK, NJ
Project Period: 1993/09/30-2007/12/31

DESCRIPTION (provided by applicant): The goals of this program are to understand how the quinolones act in mycobacteria and to discover ways to protect the compounds from the development of resistance. Previous work showed that structure modifications at the C-8 position of fluoroquinolones increase antibacterial activity, particularly with fluoroquinolone-resistant mutants. This feature, plus other variations in fluoroquinolone structure, will be examined to explore the hypothesis that lethal activity arises in part from the dissociation of gyrase subunits attached to cleaved DNA. Since gyrase subunit dissociation is assayed as lethal activity in the absence of protein synthesis, this work may reveal ways to improve action against nongrowing bacteria. To define how low fluoroquinolone concentrations affect the development of resistance, non-gyrase resistance mutants of *Mycobacterium tuberculosis*, obtained through selective growth at low drug concentration, will be examined for their ability to increase the frequency at which subsequent gyrase mutants are selectively enriched. This portion of the study is expected to influence fluoroquinolone dosing strategies. In patients, *M. tuberculosis* develops resistance so readily that anti-tuberculosis agents are administered as combination therapies; consequently, the lethal activity of new fluoroquinolones will be examined in combination with traditional agents to identify combinations of compounds that are unlikely to have intrinsic interfering activities. Traditional agents will also be combined with C-8-methoxy fluoroquinolones in a dynamic in vitro model to examine the effect of pharmacodynamic mismatch on the development of resistance. These two aspects of the program will help optimize the use of new fluoroquinolones. To provide a clinical context for the work, isolates from New York City will be examined for susceptibility to fluoroquinolones. Comparison of isolates obtained in the early 1990s with those obtained recently will indicate whether susceptibility is being lost. Principles emerging from these in vitro studies may be generally applicable to bacterial infections for which fluoroquinolone treatment is indicated; ideas concerning resistance may also extend to other compounds in which de novo antimicrobial resistance develops in a gradual, stepwise manner.

Grant: 2R01AI035272-12
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: KOLATTUKUDY, PAPPACHAN E PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: Molecular Biology of Unique Wall Lipids of Mycobacteria
Institution: UNIVERSITY OF CENTRAL FLORIDA ORLANDO, FL
Project Period: 1993/09/30-2007/12/31

DESCRIPTION (provided by applicant): Tuberculosis remains the leading cause of preventable deaths. Natural spread of multidrug resistant tuberculosis and the potential use of such strains by terrorists make discovery of new targets for antimycobacterial therapy a very critical need. Cell wall lipids constitute a major physical and chemical defensive barrier that helps the pathogen evade the host defenses and antimycobacterial drugs. Therefore, synthesis of such unique lipids that are critical for infection can be suitable targets for new antimycobacterial drugs. Multiple methyl branched lipids such as dimycocerosyl phthiocerol (DIM) have been shown to be virulence factors. Elucidation of the biochemical reactions and the nature of the enzymes involved in the biosynthesis of such virulence factors is required for developing novel drugs. To this end we propose to: 1. Elucidate the functions of lipase genes and their possible role in pathogenesis, a) Express and characterize the catalytic capabilities of lip gene products and determine whether disruption of specific lip genes will result in the absence of a specific class of acyl-lipids or absence of a specific group of esterified fatty acids, and determine whether the mutations affect virulence, b) Directly test whether the expressed lip gene products can release the acyl chains from the expressed synthases in vitro. 2. Elucidate the biological function of tes genes and their possible role in virulence, a) Express tes genes and characterize the catalytic capabilities of their products, b). Disrupt the three tes genes, determine the effects on lipid metabolism by using ¹⁴C-labeled acetate and ¹⁴C-labeled propionate as radiotracers and determine the effect of the mutations on virulence of the pathogen in the murine model. 3. Elucidate the function of wes genes, a) Express the wes genes and test whether these gene products are involved in the esterification of the methyl branched acids generated by the large synthases to the hydroxyl groups in the ultimate acceptors, b) Determine the consequences of disrupting the wes genes on lipid metabolism and test the virulence of mutants that show novel biochemical phenotypes. 4. a) Elucidate the role of the novel (subunit in the enzyme that catalyzes the synthesis of methylmalonyl-CoA, the building block for the multiple methyl branched virulence factors, b) Determine whether disruption of accD4 and accD5 affect propionyl-CoA carboxylation, and methyl branched lipid synthesis in M. tuberculosis and its virulence. c) Determine the possible role of succinate as a source for methylmalonyl-CoA. The results of this study are likely to give information and tools for screening chemical libraries to discover new types of drug candidates directed at novel targets in M. tuberculosis and thus help in combating multidrug resistant tuberculosis.

Grant: 2R01AI035717-06A2
Program Director: HALL, ROBERT H.
Principal Investigator: TRUCKSIS, MICHELE MD
Title: Accessory cholera enterotoxin, ACE, Mechanism of action
Institution: UNIV OF MASSACHUSETTS MED SCH WORCESTER, MA
WORCESTER
Project Period: 1995/09/15-2007/01/31

DESCRIPTION (provided by applicant): Colonization of the small intestine by *Vibrio cholerae* causes the potentially lethal disease cholera due to massive salt and water secretion. Although the dehydrating diarrhea of cholera is attributed to secretion stimulated by cholera toxin, two other toxins of *V. cholerae* that alter short circuit current or resistance in Ussing chambers have been identified. *V. cholerae* ACE (accessory cholera enterotoxin) was initially described in 1993 as a toxin which increased short circuit current and potential difference in rabbit ileum mounted in Ussing chambers and caused fluid secretion in ligated rabbit ileal loops. We have investigated the mechanism of action of ACE utilizing monolayers of polarized intestinal epithelial cells (T84 cells) mounted in modified Ussing chambers. In these studies we identified novel physiology stimulated by ACE including that ACE is dependent on calcium as a second messenger; that although it is a calcium-dependent agonist it is unique in that it has a prolonged current response and acts synergistically with other calcium-dependent agonists; and finally, that it stimulates secretion that is equally dependent on chloride and bicarbonate ions (a newly emerging mechanism of secretion). We hypothesize that ACE interacts with the polarized intestinal epithelial cell, through a receptor present on the apical surface and that through this interaction it initiates signal transduction with calcium as a second messenger. We also hypothesize that ACE potentiates the Cl⁻ secretory activity of carbachol by blocking the normal inhibitory pathway stimulated by carbachol. The Specific Aims are 1) Identify the ACE receptor; 2) Examine the signal transduction pathways stimulated by ACE; and 3) Examine the interaction of ACE with calcium-mediated inhibitory pathways. We will use molecular genetic, biochemical and cell physiology methods to examine the mechanism of action of ACE. The long term objectives of this proposal are to enhance our understanding of the role of ACE in cholera pathogenesis and to identify novel mechanisms of action of bacterial toxins. The utility of ACE in investigating the epithelial transport pathways in the intestine, and perhaps other tissue types lies in ACE's novel physiology. The characterization of the ion channels activated by ACE may provide valuable information, which can be used to develop pharmacological modulators of chloride and bicarbonate secretion useful in the treatment of diarrheal diseases (excessive secretion) or cystic fibrosis (defective secretion).

Grant: 2R01AI037027-09
Program Director: PERDUE, SAMUEL S.
Principal Investigator: LEE, CHIA Y
Title: Genetic regulation of staphylococcal capsule
Institution: UNIVERSITY OF KANSAS MEDICAL CENTER KANSAS CITY, KS
Project Period: 1994/12/01-2007/11/30

DESCRIPTION (provided by applicant): Staphylococcus aureus is a major human pathogen, which can cause serious infections. The organism has become increasingly problematic recently due to emerging multiple-drug resistance strains that are unresponsive to antibiotic treatment. New methods of treatment are therefore urgently needed. Almost all strains of S. aureus produce capsular polysaccharide, which have been classified into 11 serotypes. Type 5 and 8 capsules, which are structurally and genetically similar, are the predominant capsules and thus have been used as targets for vaccine development. Recent studies, including our own, have shown that these capsules act as antiphagocytic virulence factors and thereby play an important role in staphylococcal pathogenesis. The production of these capsules are influenced by environmental stimuli, however, very little is known about their regulation at the molecular level. Recently, we have characterized the promoter region controlling the expression type 8 capsule genes and have shown that type 5 and 8 capsules are regulated by several known regulators. We have also genetically identified several putative regulatory genes. These results therefore suggest that type 5 and 8 capsules are regulated by a complex regulatory network. In this proposal, we will focus on the regulatory aspect of capsule expression. We propose to accomplish three specific aims: (i) to characterize the regulatory genes involved in the control of capsule production by molecular approaches; (ii) to study the interactions between regulators by genetic and biochemical analyses of mutants deficient in regulatory genes to elucidate regulatory networking; (iii) to investigate the regulation of capsules by environmental stimuli by focusing on carbon dioxide effect both in vitro and in vivo. The successful completion of the studies outlined in this application will undoubtedly unveil the regulatory mechanism controlling the expression of these important virulence factors, which will provide a firm basis for developing new methods of treating staphylococcal infections.

Grant: 2R01AI037744-06A2
Program Director: PERDUE, SAMUEL S.
Principal Investigator: SAMUEL, JAMES E PHD
Title: Pathogenic Roles of *Coxiella burnetii* Proteins
Institution: TEXAS A&M UNIVERSITY HEALTH SCIENCE COLLEGE STATION, TX
CTR
Project Period: 1995/07/15-2007/12/31

DESCRIPTION (provided by applicant): *Coxiella burnetii*, the etiologic agent of Q fever, is an obligate intracellular bacteria that replicates within an apparently unmodified phagolysosome. Among intracellular pathogens the organisms are novel in their location of replication, extreme stability to stress, and persistence in the environment. Bacterial replication is controlled primarily by activated macrophage/monocyte and PMN killing mechanisms stimulated by a cell mediated response, but the exact nature of these mechanisms is undefined. We propose that survival mechanisms are the principle virulence determinants of *C. burnetii*. We will evaluate four strategies for their contribution in survival. First, we will define the nature and role in survival of a *C. burnetii* lifecycle. The working hypothesis is that separable, morphological variants represent stages of cell differentiation with specific roles in intracellular and extracellular survival. Based upon earlier studies and data presented in Preliminary Studies, two major variant forms (large cell variants and small cell variants) differentially express proteins that support a model of metabolically most active dividing cells and stationary forms, respectively. Second, we will characterize the requirement for and acquisition systems used to obtain and regulate iron. The working hypothesis is that *C. burnetii* must accommodate conditions of limiting and high iron levels to survive in the phagolysosome. Data presented in Preliminary Studies demonstrate a *C. burnetii* ferric uptake regulator (*fur*) gene and proteins involved in iron acquisition using a ferric uptake regulator titration assay (FURTA). Third, we will characterize the role of anti-oxidant gene products in survival. The working hypothesis is that *C. burnetii* express enzymes which detoxify oxygen radicals outside of their cytoplasm and respond to oxidative stress by repairing DNA damage caused by oxygen radicals. Data presented in Preliminary Studies allow us to characterize catalase and RecA. We will test the molecular Koch's postulate for the requirement of RecA by creating a transdominant negative mutant through genetic transformation. Finally, we will characterize the role of acid phosphatase in intracellular survival. All specific aims will be facilitated by an ongoing genome sequence project. The working hypothesis is that *C. burnetii* express acid phosphatase which phosphorylates phagolysosomal proteins and reduces their antibacterial activity.

Grant: 2R01AI038901-06A1
Program Director: PERDUE, SAMUEL S.
Principal Investigator: BAYLES, KENNETH W PHD
Title: The molecular control of bacterial autolysis
Institution: UNIVERSITY OF IDAHO MOSCOW, ID
Project Period: 1997/07/01-2007/12/31

DESCRIPTION (provided by applicant): Studies of the *Staphylococcus aureus* *lytSR* regulatory locus have led to the identification of a complex regulatory system that controls the activity of peptidoglycan hydrolases produced by the cell. One component of this regulation is the *IrgAB* operon whose expression has been shown to inhibit murein hydrolase activity and cause tolerance to penicillin. It has been proposed that the products of this operon function in a manner analogous to bacteriophage-encoded antiholins, which are membrane-associated proteins that inhibit peptidoglycan hydrolase activity at the posttranslational level. Recently, homologous proteins encoded by the *S. aureus* *cid* operon have been identified and studied. Preliminary experiments indicate that this operon encodes the holin component of this system that enhances peptidoglycan hydrolase activity and increases sensitivity to penicillin. The possibility that the *Irg* and *cid* operons are involved in a bacterial programmed cell death (PCD) mechanism has been indicated since the *cid* mutant exhibits tolerance to other bactericidal agents besides penicillin. These include rifampicin and mitomycin C, which have distinct cellular targets suggesting that this system responds to nonspecific cellular stress by inducing cell death. The recent finding that *cid* expression is dependent on the alternate stress response sigma factor, Sigma B, strengthens this hypothesis. The experiments described in this proposal are based on four specific aims. The first aim utilizes a genetic approach to explore the role the *cid* and *Irg* gene products during the bactericidal response to antibiotics and biocides. The second aim includes flow cytometric studies of membrane potential and cell wall pH designed to more clearly define the roles of the *cid* and *Irg* gene products in the regulation of murein hydrolase activity. Purification and analysis of the *cid* and *Irg* gene products is the third aim of this proposal with the goal of defining the interactions of these proteins prior to and during cell death. Finally, the fourth aim will study the regulation of *cid* transcription using molecular strategies to examine the kinetics of *cid* expression, the cis-acting elements necessary for normal regulation, and the role of a putative transcription activator protein. The long-term objectives of this study are to establish the roles that the *Irg* and *cid* operons play in the molecular control of bacterial PCD and to explore novel new therapeutic strategies to combat bacterial infection.

Grant: 2R01AI039129-06A2
Program Director: HALL, ROBERT H.
Principal Investigator: SACK, R B MD
Title: Epidemiology & Ecology of Vibrio Cholerae in Bangladesh
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 1996/09/01-2008/02/28

DESCRIPTION (provided by applicant): Cholera continues to be a major public health problem throughout the developing world. The primary objective of the original application was to test the hypothesis that environmental factors involving surface waters were responsible for the observed periodicity and pandemic nature of cholera. Data collected strongly suggest, but do not prove, that environmental factors are predictive of cholera outbreaks. Work will now focus on further defining the positive environmental associations that were found, and in determining, by genetic methodology, the relatedness of *V. cholerae* strains isolated from the environment and those from patients. Work will continue in Bangladesh in association with the Centre for Health and Population Research (ICDDR, B), the University of Maryland Biotechnology Institute and School of Medicine, and Emory University. Bi-weekly clinical and environmental surveillance will continue at two of the original sites where cholera outbreaks were frequent and in a new third site (Sunderbans), an area of mangrove swamps close to the Bay of Bengal, the site of entry for the O139 vibrios in Bangladesh in 1992. Improved assays will focus on: (1) identifying and culturing *V. cholerae* in surface waters, using both standard techniques and colony blots with non-radioactive DNA probes, (for *V. cholerae* species, O1, O139, ctx, and tcpA) DNA extraction, and PCR, (2) assessing the relationship between clinical and environmental isolates by genotyping (using AFLP, ERIC-PCR, and MLST) and (3) identifying and enumerating *V. cholerae* attached to specific zooplankton species, using direct fluorescent antibody techniques and fluorescent in situ hybridization assays. Using the surveillance data our model of cholera transmission will be further refined. This will be useful in predicting outbreaks of cholera, not only in Bangladesh, but also in other countries with similar aquatic environments, thereby allowing early mobilization of preventive and treatment measures. Bangladesh is unique in affording the environment for doing these types of studies.

Grant: 2R01AI039499-06A2
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: DEAN, DEBORAH A MPH
Title: Pathogenesis of Persistent Chlamydial STDs
Institution: CHILDREN'S HOSPITAL & RES CTR AT OAKLAND, CA
OAKLAND
Project Period: 2003/09/15-2007/12/31

DESCRIPTION (provided by applicant): Chlamydia trachomatis(CT) is the leading cause of sexually transmitted diseases (STD) in the developed world. CT infections and their sequelae of pelvic inflammatory disease, ectopic pregnancy, and infertility are responsible for approximately 80% of the estimated \$2.5 billion annual cost of these infections in the United States. Further, up to 50% of women become reinfected and are at increased risk for these sequelae. Many reinfections reflect persistence that likely plays an important role in pathogenesis. The major outer membrane protein is considered to be the immunodominant protein of CT. However, the discovery of open reading frames predicted to encode a nine-member polymorphic membrane protein (Prop) gene (pmp) family in the recently published genome sequence of CT serovar D suggest that these Props may also be important in chlamydial biology. Further, CT contains a partial tryptophan biosynthesis operon (trpR, trpA, trpB) not found in a CT mouse strain (MoPn) or other species of Chlamydia. Tryptophan is essential for chlamydial replication, and tryptophan depletion in vitro results in chlamydial persistence. Our hypothesis is that the prop and tryptophan genes may undergo selection that results in differential expression or activity of these proteins that: 1) consequently determine active or persistent infection; and 2) are significantly involved in pathogenesis as an outcome of persistence or outcome of other factors. By analyzing the genetic profile of prototype and serial recurrent and persistent CT STD patient strains and by correlating these data with epidemiologic and clinical findings, we hope to identify the genes, genetic/protein variation and evolution of this variation in the organism, and how these are linked to persistence and pathogenesis. Thus, this grant will answer broad questions about the genetic and protein basis for persistence and for pathogenesis, and provide important research tools including a Database and DNA microarray that will be of long-term benefit to investigators in the field of Chlamydia. The Specific Aims for this grant are to: 1) Sequence the nine pmps, and trpR, trpA, and trpB genes for the 19 prototype serovars of CT and create a DNA microarray for these genes and ompA to differentiate strains of CT, and for use in Aim 2; and 2) Identify polymorphisms in and protein expression of the nine props, specific tryptophan operon genes, and other constitutively expressed genes among serial cervical samples from patients with persistent versus non-persistent CT STDs; correlate the genetic and protein expression profiles of these serial samples with epidemiologic and clinical findings.

Grant: 2R01AI039576-06A2
Program Director: KORPELA, JUKKA K.
Principal Investigator: KASPER, DENNIS L MD INTERNAL
MED:INFECTIOUS DISEASE
Title: PATHOGENIC MECHANISMS OF ANAEROBES IN SEPSIS
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 1997/05/01-2007/12/31

DESCRIPTION (provided by applicant): Abscess formation is a classic host response to bacteria during sepsis. Certain bacterial species, particularly *Bacteroides fragilis* and *Staphylococcus aureus* are predisposed to induce abscesses. The essential bacterial virulence factor required for abscess induction by these two pathogens is a capsular polysaccharide with a zwitterionic charge motif. Zwitterionic polysaccharides (ZPS) induce the host to form abscesses by their ability to activate T cells initiating a proinflammatory Th1 cytokine response. In contrast to the immunologic paradigm defining polysaccharides as T cell independent antigens, ZPS activate T cells in vitro as well as in vivo when incubated with antigen presenting cells (APC). There is currently no immunologic model that describes how purified polysaccharides can activate T cells. ZPS are internalized and can be detected in lysates of intracellular vesicles from the APC. Blocking of endosomal acidification results in the failure of ZPS to activate T cells. ZPS recovered from endosomal vesicles has a substantially reduced molecular size, indicating processing. We have demonstrated that MHC class II DR appears to be the molecule used by the APC to present ZPS to the T cell and that TCR alpha beta(is required for T cell activation. We hypothesize that ZPS are internalized and cycle through the APC, and that this process is required for presentation of the ZPS to the T cell. We intend to define a novel immunologic paradigm that describes how an important class of biologic molecules (carbohydrates) is recognized by the cell-mediated immune system. This will be done by investigating the cellular pathway by which ZPS cycle through the APC and activate CD4+ T cells. We have defined four specific aims: 1) Determine how ZPS are altered within the endocytic pathway and define the molecular requirements for ZPS-mediated T-cell activation; 2) Investigate the vesicular trafficking and intracellular interactions of ZPSs in the endocytic pathway; 3) Characterize the binding interactions of the MHC class II DR molecule with ZPS; 4) Determine whether T-cell activation results from "processed antigen" presentation or superantigen presentation and whether the processing of ZPS uses the same pathway as protein processing. The delineation of a mechanism for carbohydrate processing and presentation has broad relevance to the fields of microbiology and immunology and could lead to new concepts for enhancing T cell recognition of other polysaccharides.

Grant: 2R01AI040198-06
Program Director: RUBIN, FRAN A.
Principal Investigator: KOTB, MALAK PHD
Title: Immunopathogenesis of Group A Streptococcal Infection
Institution: UNIVERSITY OF TENNESSEE HEALTH SCI MEMPHIS, TN
CTR
Project Period: 2003/08/15-2008/02/29

DESCRIPTION (provided by applicant): The overall goal of the present application is to explore the way specific host genetic factors and specific bacterial virulence determinants interact together to influence the outcome of an important infectious disease. This paradigm is critical to explain the wide variety of potential outcomes that occur when a human host encounters a potentially pathogenic organism like Group A streptococcus (GAS), from asymptomatic colonization to mild infection to severe morbidity or mortality. The model we have developed studies GAS-induced severe systemic disease (SSD; the bacterial superantigens (SAGs) that are key players in eliciting SSD; and the host factors involved are the HLA class II molecules that present the SAGs to T cells. The pathogenic link of the host genetic and bacterial virulence factors is clear, since the HLA molecules are receptors for SAGs, presenting them to the TCR and transmitting biochemical signals into APCs. We found that the host immunogenetic makeup influences the clinical outcome of invasive GAS disease. Specific HLA class II haplotypes conferred strong protection from the severe forms of the invasive disease, while others increased the risk for SSD. We also determined the underlying mechanism for these genetic associations by demonstrating that the presentation of Strep SAGs by the class II DRB1*1501/DQB1*0602 haplotype, which was strongly associated with protection from SSD ($P=0.0007$), elicited significantly lower inflammatory responses as compared to their presentation by either risk or neutral haplotypes ($P<0.0001$). Patients with this protective haplotype mounted significantly reduced responses to the Strep SAGs (low responders) and were less likely to develop SSD. By contrast patients who lacked this protective haplotypes or had the DRB1*14/DQB1*0503 high risk haplotype were high responders to the Strep SAGs and were more likely to develop SSD. Our working hypothesis is that HLA class II association with risk/protection from SSD is the same for patients with invasive infections caused by particular GAS serotypes that are commonly isolated from invasive GAS infections and that share a similar SAG repertoire. The robust nature of our model is manifested in our initial identification human HLA haplotypes associated with high-risk and protection against GAS associated SSD. In this application, our combined bacterial and mammalian genetic approach will allow identification of how specific GAS SAG/human HLA combinations determine risk for a particular disease outcome. It is our hope that these results will inform the future design and application of specific therapeutic and vaccine strategies against GAS infection, and serve as a model for investigating the complex pathogenesis of other human bacterial infections.

Grant: 2R01AI040481-04A2
Program Director: PERDUE, SAMUEL S.
Principal Investigator: DAUM, ROBERT S MD
Title: Mechanisms of Glycopeptide Resistance in Staphylococci
Institution: UNIVERSITY OF CHICAGO CHICAGO, IL
Project Period: 1998/11/15-2007/03/31

DESCRIPTION (provided by applicant): Staphylococcus aureus is a leading cause of community and nosocomially-acquired infectious and toxin-mediated syndromes, some life threatening, that affect patients of all ages. The glycopeptides (GP) have been the most reliable alternatives for the therapy of S. aureus isolates that are resistant to methicillin, cross resistant to all beta-lactams, and often resistant to a wide spectrum of unrelated antimicrobials. However, the effectiveness of GPs has been eroded by the increasing recognition of resistant isolates. Our ongoing studies are aimed at identifying GP resistance mechanisms. Despite the description of numerous phenotypic and biochemical characteristics among resistant isolates, the mechanism(s) of GP resistance in S. aureus has remained incompletely defined. Available data suggest that acquisition of the resistance phenotype involves cell wall reorganization; pleiotropic changes have been documented such as altered peptidoglycan structure, coagulase activity, binding of vancomycin, autolytic activity and lysostaphin susceptibility. However, it seems unlikely that a single mechanism or sequence of mechanisms will account for resistance in all clinical glycopeptide-resistant isolates studied to date since no phenotypic or biochemical change has been uniformly found. We believe that the resistant phenotype involves multiple genetic changes. We plan to investigate the mechanism(s) of resistance with a multi-pronged approach. First, with the complete genomic sequence of four S. aureus isolates at hand, we will employ microarray analysis to compare expression patterns of relevant cell wall metabolic and 2-component signal transduction genes between GP-susceptible and resistant isolates. The availability of isogenic susceptible and resistant clinical isolate pairs will provide invaluable tools for this analysis. Appropriate up and down regulated genes will be targeted for further investigation including sequence comparison, Northern blot analysis, allelic inactivation and overexpression in relevant genetic backgrounds. These studies should lead to an understanding of the mechanisms by which S. aureus resist the bactericidal effect of GPs and hopefully can identify new ideas regarding therapy of infections caused by resistant isolates.

Grant: 2R01AI040635-06A2
Program Director: KORPELA, JUKKA K.
Principal Investigator: HUANG, SHENG-HE MD
Title: Escherichia coli invasion of brain endothelial cells
Institution: CHILDREN'S HOSPITAL LOS ANGELES LOS ANGELES, CA
Project Period: 1997/07/01-2007/12/31

DESCRIPTION (provided by applicant): E. coli K1 is the most common gram-negative organism causing neonatal meningitis. One of the least understood issues in the pathogenesis of this disease is how circulating E. coli K1 crosses the blood-brain barrier (BBB) to cause brain injury. We have demonstrated in the in vitro and in vivo models of the BBB that E. coli K1 invasion is a complex process mediated by multiple E. coli invasion determinants, among which the 50-kDa protein encoded by *ibeA* is unique to E. coli K1. A bovine IbeA-binding protein (IbeABP) has been isolated by IbeA-affinity chromatography from brain microvascular endothelial cells (BMEC). The *ibeA* locus is a genetic island of meningitic E. coli (GimA) containing 15 genes, which consist of four operons, *ptnIPKC*, *cglDTEC*, *gcxKRCI* and *ibeRAT*. IbeA is an important virulence factor contributing to E. coli K1-mediated invasion in BMEC and the 14 other genes may involve in energy metabolism. The regulatory protein IbeR carrying an as4-interaction domain belongs to the NtrC/NifA family of transcriptional activators. It has been shown that anaerobic growth and glucose greatly enhanced E. coli K1 invasion of BMEC. Based on these results, we have hypothesized that the *ibeA* locus-mediated invasion and energy metabolism are coordinate events in pathogenesis of E. coli K1 meningitis. Interactions between IbeA and its receptor induce cellular responses that are required for E. coli entry of BMECs. Invasion gene expression is regulated by the genetic island GimA including IbeR and environmental signals. Our hypothesis will be tested through the following Specific Aims. (1). To examine how IbeA contributes to E. coli K1 entry of human BMECs. (2). To further characterize the role of IbeABP in E. coli K1 invasion of human BMEC. (3). To determine how E. coli K1 invasion gene expression is regulated by the *ibeA* locus (GimA) including IbeR and environmental signals (glucose and oxygen tension).

Grant: 2R01AI040689-06A1
Program Director: PERDUE, SAMUEL S.
Principal Investigator: SAHNI, SANJEEV K PHD
Title: Rickettsia-induced transcriptional activation
Institution: UNIVERSITY OF ROCHESTER ROCHESTER, NY
Project Period: 1997/08/01-2008/01/31

DESCRIPTION (provided by applicant): *Rickettsia rickettsii*, an obligate intracellular bacterium and etiologic agent of Rocky Mountain spotted fever, infects and proliferates predominantly within vascular endothelial cells, which respond by activating a series of distinct signal transduction pathways. *R. rickettsii* infection of endothelial cells results in the activation of nuclear factor-kappaB (NF-kappaB), a transcription factor which controls the expression of an array of genes involved in bacterial infections, immune response, and apoptosis. The anti-apoptotic functions of NF-kappaB are critical for the protection of host cells from apoptotic death during *R. rickettsii* infection. The goal of this application is to further our understanding of signaling mechanisms underlying *Rickettsia*-induced transcriptional activation, to evaluate their participation in the host cell response to infection, and to investigate if interfering with these signals affects rickettsial replication. Aim 1 will characterize the activation of IkappaB kinase complex (IKK) and phosphorylation/degradation of IkappaB proteins during infection. We will determine the kinetics of activation of catalytic subunits, IKKalpha and IKKbeta by an immunoprecipitation (IP): kinase assay. The role of the regulatory subunit, IKKgamma, will be evaluated using a specific, cell permeable peptide, which blocks its association with the IKK complex. The effects of selected, specific inhibitors of IKK and NF-kappaB on replication of *Rickettsia* organisms will also be studied. Aim 2 will investigate the activation of mitogen activated protein (MAP) kinases and their involvement in rickettsial invasion of endothelial cells and activation of NF-kappaB. Modulation of MAP kinase cascades, ERK1/2 and p38, will be examined by western blotting and immunostaining using phosphorylation state specific antibodies and activity assays by IP:western analysis. Aim 3 will define the regulation of chemokine induction in response to infection and explore its dependence on the MAP kinase and NF-kappaB pathways. Using specialized techniques of molecular biology and microscopy and species/strains of *Rickettsia* with varying pathogenicity, we will investigate the correlation between infection, activation of IKK/NF-kappaB and MAP kinases, and induction of chemokine response. These studies will offer important perspectives in our understanding of rickettsial pathogenesis and may lead to the identification of novel targets for supplemental chemotherapy.

Grant: 2R01AI040694-06A1
Program Director: KORPELA, JUKKA K.
Principal Investigator: SWANSON, MICHELE S PHD
Title: Evasion of Macrophage Lysosomes by L pneumophila
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 1996/12/01-2007/12/31

DESCRIPTION (provided by applicant): Innate and adaptive immune responses are initiated by macrophage phagocytosis, a complex cellular pathway that is not yet fully understood. To investigate how phagosome maturation is governed, the bacterial pathogen *Legionella pneumophila* can be exploited as a genetic probe of macrophage function. Previous studies support the hypothesis that *L. pneumophila* blocks phagosome-lysosome fusion by expressing on its surface at least two activities that are developmentally regulated. Knowledge of when, how and where inhibitors of phagosome maturation are expressed by *L. pneumophila* will be applied to identify the corresponding molecules. Factors on the bacterial surface that block phagosome-lysosome fusion will be sought genetically by isolating and characterizing mutations that affect either persistence of dotA mutants in macrophages, lectin binding, or hydrocarbon binding, and biochemically by comparing the profiles of surface proteins and carbohydrates of virulent and avirulent strains. Factors expressed by post-exponential phase bacteria to block fusion with lysosomes will also be identified by loci whose constitutive expression rescues a regulatory mutant from lysosomal killing. By understanding how *L. pneumophila* evades clearance by macrophages, one can deduce how these phagocytes routinely engulf, digest, and display foreign material, then recruit specialists to eliminate potential threats. Because macrophages are central effector cells in both humoral and cell-mediated immunity, a molecular description of their membrane traffic is likely to provide a myriad of opportunities for improving delivery of therapeutics to prevent and treat a variety of human diseases.

Grant: 2R01AI041010-06A1
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: BASEMAN, JOEL B PHD
MICROBIOLOGY:MICROBIO
OGY-UNSPEC
Title: BIOLOGY AND PATHOGENICITY OF MYCOPLASMA GENITALIUM
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR SAN SAN ANTONIO, TX
ANT
Project Period: 1996/07/01-2008/01/31

DESCRIPTION (provided by applicant): Mycoplasmas cause a range of acute and chronic infections in humans. Mycoplasma genitalium is implicated as an emerging etiological agent of sexually transmitted and respiratory diseases and arthritides in humans and has been isolated from urethral, airway and synovial specimens. M. genitalium adheres to and colonizes host cell surfaces and establishes intracellular residence by mechanisms that remain poorly defined. However, we have shown that the distinct tip attachment organelle of M. genitalium is comprised of adhesins and adherence-related proteins, which are similar but not identical to those of Mycoplasma pneumoniae. Overall, very little is known about the biology and pathogenicity of M. genitalium. Our long-term goal is to delineate the mechanisms of cytoadherence and invasion of M. genitalium and understand the dynamics of the M. genitalium-host interplay. In the Progress Report we describe ongoing studies of MG218 (tip organelle, cytoadherence-accessory protein) and its associated operon and identify previously undetected, yet seemingly important M. genitalium mucin (Mn) binding proteins (MnBPs). Mucus, which contains Mn in high quantities, is a common substance in genitourinary, gastrointestinal and respiratory tracts, and the occurrence of MnBP in M. genitalium provides a novel virulence determinant. Furthermore, we have observed differential gene expression by M. genitalium in response to host cells and their products. These research developments suggest that mycoplasma-host interactions are more intricate than previously envisioned, and our primary goal is to delineate potential pathogenic mechanisms with the following specific aims: 1. Characterize genes and gene products of the mg218-associated operon as they influence mycoplasma adherence and invasion; 2. Investigate the role of M. genitalium MnBPs in pathogenesis; and 3. Identify mycoplasma genes participating in parasitism using DNA microarrays and real-time RT-PCR. We believe that the proposed work is innovative and capitalizes on the latest developments in genomics, particularly global analysis of gene expression in DNA microarray. It also takes advantage of our recently developed methods to disrupt genes in mycoplasmas through homologous recombination. It is our expectation that the proposed studies will significantly increase our understanding of mycoplasma pathogenicity and advance new therapeutic strategies to control mycoplasma infections and disease progression.

Grant: 2R01AI041108-06
Program Director: PERDUE, SAMUEL S.
Principal Investigator: GILMORE, MICHAEL S AB
Title: Enterococcus cytolysin: Regulation and structure
Institution: UNIVERSITY OF OKLAHOMA HLTH OKLAHOMA CITY, OK
SCIENCES CTR
Project Period: 1997/12/01-2007/11/30

DESCRIPTION (provided by applicant): Enterococci are leading causes of nosocomial infections, which affect approximately 2.5 million patients in the US each year. Nosocomial infections in general, and enterococcal infections in particular, are frequently refractory to antibiotic treatment because of multiple antibiotic resistances. Few studies have examined the pathogenesis of enterococcal infection. One of the factors that has been thoroughly documented to contribute to the virulence of *Enterococcus faecalis* is the cytolysin. The cytolysin is a structurally novel toxin that consists of two dissimilar, small subunits. Each subunit is extensively post-translationally modified and proteolytically processed during maturation. Mature subunits interact with target cell membranes to effect lysis of eukaryotic cells, and killing of bacterial cells of gram-positive species. We recently described a novel, two-component regulatory system that governs expression of the *E. faecalis* cytolysin. The two regulatory components function by an unknown mechanism to shut off transcription of the cytolysin operon. Interestingly, the operon is induced by the smaller of the two toxin subunits, but only in its mature and fully processed form. Thus, the small toxin subunit must be translated, post-translationally modified, secreted, and processed through two proteolytic trimming steps before it can feed back onto the system and induce high level expression of the cytolysin operon. As the cytolysin contributes to the toxicity of enterococcal infection, it is of interest to decipher the molecular mechanisms involved in toxin regulation, and in toxin activity, toward the goal of developing a therapeutic that renders cytolytic enterococcal infections less destructive. The aims of this proposal therefore are to determine the molecular mechanism by which the two components CylR1 and CylR2 regulate expression of the cytolysin operon; and to determine how the structures of the toxin subunits CylLL and CylLS, including post-translational modifications, relate to cytolytic bactericidal and cytolysin operon inducing function.

Grant: 2R01AI041326-06A2
Program Director: SCHMITT, CLARE K.
Principal Investigator: TZIPORI, SAUL DVM VETERINARY
MEDICINE
Title: Specific Human Monoclonal Antibodies for HUS Prophylaxis
Institution: TUFTS UNIVERSITY BOSTON BOSTON, MA
Project Period: 1996/09/23-2008/02/29

This proposal is the second revised competing renewal for continuation of NIH Award #2 R01-A1-41326. Our objective is to develop an immunotherapeutic formulation for the treatment and prevention of complications associated with Stx-producing *Escherichia coli* (STEC) infections. Clinical isolates of STEC are known to predominantly produce Stx1, Stx2, and/or Stx2c. Children are particularly susceptible to development of Stx-mediated HUS. Our hypothesis is that administration of Stx-specific antibodies will prevent or modify the outcome of infection for individuals at risk of developing HUS. In the earlier awards, we have generated a panel of human monoclonal antibodies (Hu-mAbs) specific for Stx 1 or Stx2. Using the gnotobiotic piglet model, we have shown that Stx-specific Hu-mAbs neutralize Stx and prevent development of Stx-mediated complications. We now wish to determine which Hu-mAbs should be included in a formulation suitable for clinical evaluation. Based on superior efficacy, four Hu-mAbs specific for Stx2 (3 against the A subunit and 1 against the B subunit), and 2 for Stx1 (both against B subunit) have been selected as candidates. The next step is to determine which combination of Hu-mAbs, is both compatible and highly effective. In this proposal we plan to define the structural and functional characteristics, which facilitate protective efficacy of Stx-1 and Stx2-specific Hu-mAbs (Specific Aim 1). Affinity and efficacy of each HumAb will then be studied against their respective toxin (Specific Aim 2). The efficacy of protection of a given antibody dose will then be determined in terms of time after bacterial infection (Specific Aim 3). Finally, combinations of Hu-mAbs specific for B subunit of Stx 1 and A or B subunits of Stx2, will be examined for relative efficacy and compatibility, to determine which is the most effective and thus suitable for clinical evaluation (Specific Aim 4). At the conclusion of these experiments we will have determined the components, and optimized the formulation of Hu-mAbs which will be recommended for testing in human patients. The Hu-mAbs will first be characterized and ranked according to their efficacy, affinity and compatibility with each other. The optimal amount of each Hu-mAb in the formulation required to provide the longest protection after bacterial infection will also be established. This is not a hypothesis-driven proposal, but an essential segment for the characterization of a promising therapy for HUS, against which currently there is no effective treatment.

Grant: 2R01AI041406-05
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: MADIRAJU, MURTY V
Title: Mycobacterium tuberculosis and Replication Initiation
Institution: UNIVERSITY OF TEXAS HLTH CTR AT TYLER, TX
TYLER
Project Period: 1997/08/01-2008/01/31

DESCRIPTION (provided by applicant): Human tuberculosis caused by Mycobacterium tuberculosis is the most prevalent and deadly bacterial infectious disease worldwide. This problem is compounded by the emergence of strains of M. tuberculosis that are resistant to one or more anti-tuberculous drugs. Following initial infections, M. tuberculosis frequently enters a latent or dormant state for extended periods and subsequently, under appropriate conditions or following immune suppression, revives, multiplies and causes a secondary infection. DNA replication constitutes an important step in the exit from latency. The development of novel therapeutic agents to control M. tuberculosis infections in HIV infected patients as well as other individuals is severely hindered by our limited understanding of the initiation and regulation of M. tuberculosis DNA replication and its coordination with other events in cell cycle. Initiation of DNA replication is believed to be triggered when DnaA, the putative initiator protein, interacts with oriC or origin of replication. Although oriC is essential for survival, some clinical strains of M. tuberculosis appear to tolerate major deletions and IS6110 insertions in their oriC, thereby raising questions as to how these clinical strains replicate their genome. Our research proposal focuses on understanding the replication initiation process in M. tuberculosis. Specifically, we propose to inactivate oriC, dnaA individually and together by homologous recombination in an attempt to determine whether replication in M. tuberculosis can proceed from alternate origins, and if so whether dnaA function is required for such replication. The interactions of DnaA with replication origins and consequences of these interactions will be investigated using biochemical and genetic approaches. To begin identifying the factors that could potentially affect DnaA activity, a proteomic approach combining two-dimensional gel electrophoretic separations of proteins with subsequent identification of protein spots by matrix-assisted laser ionization desorption/ionization mass spectrometry, will be used. Defining the molecular events involved in the initiation and regulation of replication is an essential prerequisite for developing defined systems for identifying novel antimycobacterial compounds, and thereby preventing the development of potentially lethal infections of M. tuberculosis.

Grant: 2R01AI041440-06
Program Director: PERDUE, SAMUEL S.
Principal Investigator: FIKRIG, EROL MD MEDICINE
Title: Immunopathogenesis of Granulocytic Ehrlichiosis
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 1998/06/01-2008/05/31

DESCRIPTION (provided by the applicant): Human granulocytic ehrlichiosis (HGE) is an emerging tick-borne infection that has now been well documented in the Northeastern and upper Midwestern United States, and in Europe. The causative agent (recently renamed *Anaplasma phagocytophila*) is an obligate intracellular pathogen that persists within neutrophils. One of the major functions of neutrophils is to eradicate microbes, and the respiratory burst - initiated by NADPH oxidase - is a major antimicrobial defense mechanism. The goal of this proposal is to understand the pathogenesis of HGE - using in vitro and in vivo models. In particular, our recently published report demonstrates that *A. phagocytophila* inhibits the respiratory burst by selectively down-regulating gp91phox, a major subunit of the NADPH oxidase holoenzyme. We will now explore this finding in detail. (a) First, we will characterize the respiratory burst during *A. phagocytophila* infection in vitro, using selected promyelocytic cell lines (HL-60, 32D and MPRO), and human neutrophils, and in vivo using a murine model of HGE and clinical samples from patients with HGE. (b) Secondly, we will examine the specific role of gp91 phox down-regulation by *A. phagocytophila* in the prevention of NADPH oxidase activity and begin to delineate the mechanism(s) of gp91 phox repression by *A. phagocytophila*. Our preliminary data strongly suggest that *A. phagocytophila* infection delays the release of CCAAT-displacement-protein (a potent repressor of gp91 phox transcription) from the gp91 phox promoter, and we will examine this further. Finally (c) we also believe that transcriptional inhibition of gp91phox cannot fully account for complete inhibition of the respiratory burst (in part because neutrophils have some preformed GP91phox protein on the cell membrane). Therefore we will also explore additional mechanisms by which *A. phagocytophila* influences formation of the active NADPH oxidase complex. These studies should lead to a greater understanding of HGE, an important new tick-borne disease, and the mechanisms that pathogens may use to interfere with neutrophil function.

Grant: 2R01AI041611-05A2
Program Director: HALL, ROBERT H.
Principal Investigator: CHOPRA, ASHOK K PHD
Title: Cytotoxic Enterotoxin in Aeromonas-mediated Diseases
Institution: UNIVERSITY OF TEXAS MEDICAL BR GALVESTON, TX
GALVESTON
Project Period: 1997/07/01-2007/02/28

Aeromonas is an emerging human pathogen, which causes gastroenteritis and septicemia. The organism is being isolated in increasing numbers from food and water, and is becoming resistant to chlorination in water and to multiple antibiotics. The focus of this grant is on a cytotoxic enterotoxin (Act) of Aeromonas, which, in addition to causing gastroenteritis, leads to fatal, non-intestinal infections. Based on the data generated during the current funding period, the following specific aims will be addressed. In Aim 1, we will identify a protein/glycoprotein receptor, which appears to be attached to the plasma membrane by a GPI-anchored protein on the intestinal epithelial cell line (T84), to which Act binds, to initiate a signal transduction cascade. These studies will be accomplished by photoaffinity labeling the receptor with Act, by a genetic approach based on a yeast two-hybrid system, and/or by surface plasmon resonance. In Aim 2, we will delineate the signal transduction cascade triggered by binding of Act to its cell surface receptor on the host cell to better understand the mechanism of action of Act. These studies will involve examining the effect of calcium mobilization and oxidative-stress pathways on TNF α and PGE2 production in Act-stimulated cells. In Aim 3, the intracellular trafficking of Act in the host cell will be examined by using specific inhibitors and the mutated Rab proteins using immunofluorescence/confocal microscopy, and we will study stress-associated protein kinase activation by Act from within the host cell. For these studies, Act will be delivered into T84 cells via lipofection or electroporation to prevent receptor-mediated signaling. In Aim 4, we will dissect the role of various mediators generated via Act signaling that lead to different biological effects of Act. We will specifically examine mechanism of Act-induced apoptosis in macrophages via caspase 9 and the role of various biological mediators in fluid secretion, using both in vitro and in vivo models. These studies will provide valuable information in intervening in the severe pathological sequelae associated with Aeromonas infections in the future.

Grant: 2R01AI042081-05A1
Program Director: TAYLOR, KATHERINE A.
Principal Investigator: SOLNICK, JAY V MD
Title: Gene expression during H. pylori-host interactions
Institution: UNIVERSITY OF CALIFORNIA DAVIS DAVIS, CA
Project Period: 1997/12/01-2007/12/31

DESCRIPTION (provided by applicant): Helicobacter pylori causes an inflammatory infiltrate in gastric mucosa that in about 10% of cases progresses to peptic ulcer disease or gastric cancer. Disease results from an interaction between strain-specific bacterial virulence genes and the particular host response, neither of which is well understood. Since experimental inoculation of rhesus macaques with H. pylori causes gastritis that closely mimics human infection, this model provides a unique opportunity to further our understanding of H. pylori pathogenesis. Rapid progress in genomics and gene expression technologies makes it possible to use the macaque model to study the H. pylori host-pathogen interaction by in vivo analysis of gene expression. We propose to extend our work in the rhesus model of H. pylori into an analysis of bacterial (Specific Aim 1) and host (Specific Aim 2) gene expression during experimental infection. Monkeys will be inoculated with a wild type H. pylori strain that reproducibly infects macaques, or with an isogenic mutant deleted in a specific gene implicated in H. pylori pathogenesis. Since pH is fundamental to host gastric physiology and to the niche in which H. pylori thrives, bacterial and host gene expression will also be examined after pharmacological manipulation of gastric pH. Quantitative real-time RT-PCR will be used to examine expression of H. pylori gene families that are likely involved in H. pylori-related disease or immune evasion. Gene expression in bacterial cells grown in vitro will be compared to that in cells isolated directly from infected monkeys. DNA microarray analysis will be used to study host expression of genes thought to be important in the fundamental processes of inflammation, proliferation, apoptosis, and cell signaling. Since the host immune response is increasingly recognized as a critical variable in the outcome of infection, we will also study host gene transcription after immunization with urease coupled with either CpG or alum adjuvant, in order to promote aTh1 or Th2 immune response, respectively (Specific Aim 3). These studies will provide a functional genomic understanding of the H. pylori host-pathogen relationship that may have implications for novel treatment or vaccine strategies.

Grant: 2R01AI042156-06
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: STEPHENS, RICHARD S PHD
Title: Virulence Determinants of Chlamydia
Institution: UNIVERSITY OF CALIFORNIA BERKELEY BERKELEY, CA
Project Period: 1997/12/01-2008/02/28

DESCRIPTION (provided by applicant): Our broad, long-term objective is to understand the molecular biology of Chlamydia with the goal of using acquired information for new therapeutic and prophylactic interventions and improved diagnostic and preventive strategies. Chlamydiae have been exceedingly difficult organisms to study from a biological or molecular genetic perspective. First, because they can only be grown within eukaryotic host cells, little is known concerning the basic biochemical and physiological pathways. Second, most chlamydial gene promoters are not efficiently recognized by *E. coli* transcriptional machinery. Third, there has not been a genetic system developed, wherein DNA can be stably introduced into these organisms. Moreover, even the selection of chlamydial mutants has not offered a general genetic approach because of their limited propagation in tissue culture, dependence upon intracellular growth and a single developmental form required for infection of mammalian cells. The lack of the ability to utilize direct genetic systems has effectively prevented an essential and powerful experimental approach from the repertoire of chlamydial research. The availability of the complete genome sequences for *C. trachomatis* and *C. pneumoniae* mitigate many of these technical research challenges because this information can be used to markedly facilitate experimental investigations. Our hypothesis is that the microbiologically unique and virulence-defining chlamydial developmental cycle is regulated at the level of transcription by signal transduction response regulators and other regulators of transcription. During the requested period of support we will test this hypothesis by: I. Map mRNA 5'-ends of developmentally regulated genes and operons. Based on transcriptional array data and prediction of operons, we will determine the 5' ends of mRNA for developmentally regulated genes or operons. This information will provide a database of chlamydial promoters and transcriptional regulatory regions required for analyzing mechanisms of developmental regulation. II. Identify and characterize genes regulated by two-component signal transduction response regulators and DNA-binding proteins. We identified only two signal transduction response regulators in the chlamydial genome, CtcB-CtcC, analogs of the NtrB-NtrC-family of response regulators and CpxR, an analog of OmpR. We will test which chlamydial genes are regulated by genome-wide array analysis for binding DNA of intergenic promoter regions. Mechanisms of gene regulation will be expanded by testing the intergenic arrays for proteins present in RB and EB that bind DNA and likely regulate transcription. III. Surrogate models of transcriptional regulation in *E. coli*. Based upon the information obtained from Aims 1 & 2, we will model chlamydial gene regulation in *E. coli* and test specific hypotheses of gene regulatory function.

Grant: 2R01AI042345-05
Program Director: BAKER, PHILLIP J.
Principal Investigator: SKARE, JON T PHD
Title: Genetic Mechanisms in *Borrelia burgdorferi* Pathogenesis
Institution: TEXAS A&M UNIVERSITY HEALTH SCIENCE COLLEGE STATION, TX
CTR
Project Period: 1999/04/01-2008/03/31

DESCRIPTION (provided by applicant): *Borrelia burgdorferi*, the etiologic agent of Lyme disease, is the most frequent arthropod borne infection in the United States. Despite the information obtained from the genome sequence, very little is understood regarding how this bacterium responds to the distinct niches it occupies in nature (arthropods and mammals) and no regulatory systems have been molecularly defined. The investigators long-term goal is to understand how *B. burgdorferi* modulates gene expression in response to environmental signals and to link this knowledge to the synthesis of specific molecules that contribute to pathogenesis. The objectives of this application are to characterize an oxygen-specific regulatory network and relate its role to the expression of genes important in the life cycle of *B. burgdorferi*. The hypothesis is that dissolved oxygen is an important cue that *B. burgdorferi* uses to sense its environment and mobilize a response via the regulatory locus *perR*. *PerR* is a metallo-regulatory protein that modulates the expression of genes involved in the oxidative stress response. *B. burgdorferi perR* mutants are resistant to hydrogen peroxide, suggesting that *PerR* represses expression of redox responsive genes in *B. burgdorferi*. The investigators also determined that the *perR* mutant is de-repressed in additional genes unrelated to the stress response, suggesting that *PerR* constitutes a regulon. The investigators propose to characterize the *PerR* regulon with the following Specific Aims: (1) Identify the genes that comprise the *PerR* regulon. The investigators will use a genomic and proteomic based approach to determine genes regulated by *PerR* in response to the redox status; (2) Determine the mechanism of *PerR* regulation. *PerR* binds metals and may be redox responsive. The investigators propose to determine which co-factors modulate *PerR* activity; and (3) Relate proteins regulated by the redox status of cells to in vivo expression, and protective immunity. Several antigens are upregulated in *perR* mutants and when oxygen levels are low. *PerR* regulated antigens expressed during periods of oxidative stress and repressed when oxygen is limiting will be tested as protective immunogens. The investigators predict that the *PerR* regulon is important for both the physiology and pathogenesis of Lyme borreliosis as it modulates the expression of genes required for resistance to toxic oxygen species and factors required for adhesion, respectively.

Grant: 2R01AI042347-06
Program Director: HALL, ROBERT H.
Principal Investigator: WALDOR, MATTHEW K MD
Title: Molecular Biology and Virulence of CTX Phage
Institution: NEW ENGLAND MEDICAL CENTER BOSTON, MA
HOSPITALS
Project Period: 1998/01/01-2003/07/31

DESCRIPTION (provided by applicant): CTXphi is a filamentous bacteriophage that encodes cholera toxin. This is the principal virulence factor of *Vibrio cholerae*, the Gram-negative bacterium that causes the severe diarrheal disease cholera. CTXphi is the first filamentous bacteriophage shown to mediate the horizontal transfer of a virulence gene. CTXphi integrates into the *Vibrio cholerae* chromosome and, in the lysogenic state, most CTXphi genes are not expressed due to the activity of the CTXphi repressor, RstR. Generally, the integrated form of CTXphi is found as part of tandem arrays of prophage DNA interspersed with the related genetic element RS1. RS1 encodes a protein, RstC, that can counter RstR repression and lead to markedly enhanced expression of CTX prophage genes including *ctxAB*, the genes encoding cholera toxin. The long-term goal of this work is to understand the molecular events in the life cycle of CTXphi and the role that this phage plays in the pathogenesis of cholera. The proposed studies will explore 3 processes central to the phage life cycle: i) the site-specific integration of phage DNA into the bacterial chromosome; ii) the repression of most phage gene expression following integration; and iii) the activation of phage gene expression and virion production by environmental and genetic stimuli. Experiments in Aim 1 to identify the mechanism and factors that mediate the integration of CTXphi DNA into the *V. cholerae* chromosome will reveal how the chromosome encoded recombinases XerC and XerD interact with phage and chromosome sequences to accomplish CTXphi integration. These studies will elucidate a novel mechanism of phage integration and may shed light on the mechanism of *ctxAB* amplification as well. Experiments in Aim 2: to characterize the regulation and mode of action of RstR will clarify how CTXphi can be maintained in a quiescent state. *rstR* autoregulation and modulation of RstR levels by environmental factors will be explored. RstR's binding to its unusual operators will also be studied. Experiments in Aim 3 to determine the mode of action of RstC will explore how RstC can inactivate RstR-mediated repression. RstC's ability to bind to either RstR and/or RstR's binding sites will be investigated and the expression of *rstC* during infection will be measured. All of these studies will yield insights into fundamental aspects of phage biology. In addition, they may reveal ways in which changes in phage gene expression or copy number can contribute to the pathogenicity of *V. cholerae*.

Grant: 2R01AI042756-05A2
Program Director: PERDUE, SAMUEL S.
Principal Investigator: ARMSTRONG, RICHARD N PHD
CHEMISTRY:CHEMISTRY-
UNSPEC
Title: ENZYMOLOGY OF ANTIBIOTIC RESISTANCE
Institution: VANDERBILT UNIVERSITY NASHVILLE, TN
Project Period: 1998/02/01-2007/12/31

DESCRIPTION (provided by applicant): In the last two decades it has become increasingly clear that the efficacy of antibiotics for the treatment of infectious diseases is in jeopardy due to the common appearance of drug resistant strains of microorganisms. Understanding the mechanisms of antimicrobial resistance is crucial for effective patient care in the clinic and essential for developing strategies to enhance biodefense against intentionally disseminated pathogens. Fosfomycin is a potent, broad-spectrum antibiotic effective against both Gram-positive and Gram-negative microorganisms. A decade after its introduction plasmid-mediated resistance to fosfomycin was observed in the clinic. Investigations supported by this project have established that the resistance is due to a metalloenzyme (FosA) that catalyzes the addition of glutathione to the antibiotic, rendering it inactive. Similar resistance elements have now been shown to exist in the genomes of several pathogenic microorganisms including, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus anthracis*, *Brucella melitensis*, *Listeria monocytogenes* and *Clostridium botulinum*. Genomic and biochemical analysis from this project suggest that there are three distinct subgroups of metalloenzymes, termed FosA, FosB and FosX, that confer resistance through somewhat different chemical mechanisms. The objectives of this research project are to identify plasmid and genomically encoded proteins involved in microbial resistance to fosfomycin and to elucidate the underlying structural and mechanistic enzymology of resistance. These objectives will be accomplished by integrating enzymological, biophysical and genomic analyses of the resistance problem. The three-dimensional structures of the FosA from *Pseudomonas aeruginosa* and its relatives FosB and FosX will be determined by X-ray crystallography. The chemical mechanisms of catalysis will be elucidated by: (i) examination of the inner coordination sphere of Mn 2+ in FosA and FosX by EPR and ENDOR spectroscopy; (ii) a steady state kinetic analysis of the thiol selectivity of FosA and FosB, and (iii) a mechanistic study of the unique hydration reaction catalyzed by FosX. Potential transition state inhibitors will be investigated by structural, spectroscopic and kinetic techniques. The thermodynamics of the interaction of substrates and inhibitors with the enzymes will be examined by isothermal titration calorimetry. Particular emphasis will be placed on the enzymes from the pathogens *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Clostridium botulinum*. The intent of this investigation is to establish the mechanistic and structural bases for the design of drugs to counter both plasmid borne and genomically encoded resistance to fosfomycin.

Grant: 2R01AI042783-06
Program Director: PERDUE, SAMUEL S.
Principal Investigator: NOVICK, RICHARD P MD
MICROBIOLOGY:MICROBIOLOGY
PHYSIOLOGY
Title: Peptide Autoinducers of Staphylococcal Pathogenicity
Institution: NEW YORK UNIVERSITY SCHOOL OF MEDICINE NEW YORK, NY
Project Period: 1998/05/15-2007/12/31

DESCRIPTION (provided by applicant): The agr locus encodes the central regulatory system for staphylococcal pathogenesis and other stress-related functions. It is a quorum-sensing system that contains a two-component signal transduction module, encoded by agrA and C, and a peptide autoinducer, encoded by agrD, that is the activating ligand. Natural variants exist that cross inhibit agr autoinduction in heterologous combinations, thus blocking pathogenesis. This is the continuation of a long-term program whose overall goal is to understand the mechanism of agr autoinduction, the role of the agr autoinduction circuit in the pathogenesis of staphylococcal disease, and the biological significance of agr variants and their biotypes. Specific Aims for this period are: 1. To determine the mechanism of autoinducing peptide biosynthesis and the mechanism by which the mature secreted peptide interacts with its receptor, including both activation by cognate peptides and inhibition by heterologous ones. 2. To determine the role of the agr system in the pathogenesis of staphylococcal disease by following the expression of specific genes in vivo, the consequences of certain mutations, and the effects of the inducing or inhibiting peptides on the course of an experimental infection. 3. To characterize the agr specificity groups for traits or genes that are shared within a group and divergent between groups, with respect to pathogenic adaptations of the organism. Design and Methods: A direct ligand-binding assay using radioactive peptide will be developed to analyze receptor-ligand interactions. Variant peptides will be synthesized to delineate the structural and sequence requirements for receptor activation and inhibition. Other variants will be synthesized to enhance stability and activity in vivo to maximize the therapeutic efficacy of inhibiting virulence. Certain bacterial genes will be fused to a luciferase reporter, which will permit the monitoring of their expression as well as of the fate and persistence of infecting organisms in a murine infection model, by means of a luciferase-detecting imaging camera. This camera will monitor the effects of in vivo agr inhibition on the infecting organisms and on gene expression in vivo. Agr group-specific biotypes will be delineated to identify group-specific traits that may be correlated with pathogenic behavior - site and type of lesion, level of virulence, antibiotic resistance, etc..

Grant: 2R01AI042806-06A1
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: ENGEL, JOANNE N MD MEDICINE
Title: Mechanism of Pseudomonas-mediated epithelial cell damage
Institution: UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA
Project Period: 1998/06/01-2008/05/31

DESCRIPTION (provided by applicant): *Pseudomonas aeruginosa* (PA) is one of the most virulent opportunistic pathogens of man. The morbidity of PA infections results from the ability of the bacterium to colonize previously injured or disrupted epithelial cell layers leading to further epithelial cell damage, inhibition of wound healing, and access to other tissues or the blood stream. Our initial work utilized a novel genetic screen to identify new virulence factors of PA required for epithelial cell injury. These studies identified new virulence factors (the type III secretion system and the secreted effector protein ExoU) and also suggested new functions in virulence for previously identified virulence factors (type IV pili). The role of these genes in pathogenesis was validated using assays testing for virulence in the tissue culture system and in a mouse model of acute pneumonia. We discovered that PA can damage epithelial cells and macrophages by at least two type III-secretion dependent pathways. The first involves ExoU-mediated necrosis and the second pathway has features of apoptosis. In this competitive renewal we will continue these studies with the long term goals of (i) understanding the complex interplay between the bacterial type III secretion system, its secreted effectors, and the host eukaryotic cell and (ii) elucidating the role of type IV fimbriae as virulence factors in acute infections caused by PA. Our short term goals will focus on (i) the pathways by which ExoT alters the host cell cytoskeleton, (ii) the mechanism of type III secretion-dependent apoptosis, (iii) and the role of type IV pill in type III secretion. Specific aim 1. We will test the hypothesis that multiple domains of ExoT contribute to its role in inhibiting bacterial internalization, inducing cytoskeletal changes and cell rounding, and inhibiting wound healing of eukaryotic cells. Specific Aim 2. We will dissect the mechanism by which type III secretion induces apoptotic like death in host cells. Specific Aim 3. We will explore the biological roles of the polarly located type IV pili in virulence. We will test the hypothesis that specific components of type IV pill are required for discrete steps in type III-mediated secretion and translocation.

Grant: 2R01AI042999-07

Program Director: SIZEMORE, CHRISTINE F.

Principal Investigator: DERETIC, VOJO P PHD MOLECULAR BIOLOG
OTHER

Title: M. tuberculosis defenses against host oxidant systems.

Institution: UNIVERSITY OF NEW MEXICO ALBUQUERQUE, NM
ALBUQUERQUE

Project Period: 1998/04/01-2007/12/31

DESCRIPTION (provided by applicant): Mycobacterium tuberculosis survives, multiplies, and, persists in infected macrophages. In this proposal, we plan to study oxidative stress response defenses in M. tuberculosis, and determine how the tubercle bacillus withstands, eludes, and interferes with systems generating reactive oxygen and nitrogen intermediates (ROI and RNI) in the host phagocytic cells. Our studies have originated from early observations that the M. tuberculosis genome has a defective gene (pseudogene) encoding a regulator of oxidative stress response, and that the expression of anti-oxidant systems is anomalous in the virulent M. tuberculosis strains. These studies have now transformed into a two-prong study of (a) systems in M. tuberculosis that detoxify ROI and RNI (with added implications for isoniazid action and the exquisite sensitivity of the tubercle bacillus to INH); and (b) the previously unappreciated mechanisms of interference with the assembly, localization, or activation of enzymatic systems responsible for the delivery of bactericidal oxygen and nitrogen intermediates into the mycobacterial phagosome. Several new observations provide the foundation for this application: (a) New evidence shows that INH activation with KatG results in the generation of NO*, known to show strong anti-M. tuberculosis action. (b) The observed alterations of the mycobacterial phagosome suggest that M. tuberculosis interferes with the assembly or activation of phagocytic NADPH oxidase via interference with phosphatidylinositol phosphate interconversions on mycobacterial phagosomes. (c) The preliminary data also show that inducible nitric oxide synthase (iNOS) is excluded from the vicinity of mycobacterial phagosome. We propose the following 3 specific aims to demonstrate the relationships listed above: 1) Investigate the unusual adaptations of M. tuberculosis oxidative stress response systems. 2) Investigate the mechanism of NO* generation during INH activation by KatG and its role in antimycobacterial action of isoniazid. 3) Investigate molecular mechanisms of M. tuberculosis interference with iNOS recruitment to mycobacterial phagosomes and with oxidative bactericidal mechanisms in macrophages. We anticipate that these findings will explain important aspects of M. tuberculosis pathogenesis and its ability to parasitize macrophages. The phenomena addressed in this proposal will provide a foundation for development of new therapies that will improve present treatments of tuberculosis.

Grant: 2R01AI043063-05A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: CABELLO, FELIPE C MD OTHER AREAS
Title: Stringent response and bmp expression in *B. burgdorferi*
Institution: NEW YORK MEDICAL COLLEGE VALHALLA, NY
Project Period: 1998/07/01-2008/02/29

DESCRIPTION (provided by applicant): In previous studies we have shown that the *B. burgdorferi* bmp chromosomal gene cluster encoding the paralogous Bmp membrane lipoproteins is present in all *B. burgdorferi* sensu lato. This cluster forms two complex transcriptional units that are transcribed into variable monocistronic and polycistronic messages to generate different bmp mRNA and gene product concentrations. Nutritional stress in *Borrelia burgdorferi* triggers the stringent response that modulates expression of many genes and is mediated by the alarmon (p)ppGpp. Our work now indicates that *B. burgdorferi* has a transcriptionally active rel/Bbu, gene needed for generation of (p)ppGpp and that the presence of (p)ppGpp is associated with the modulation of expression of bmp and other genes under different environmental conditions. The genetic, transcriptional, and biological characteristics of the bmp gene cluster makes it an ideal model to study chromosomal gene regulation both by trans regulators such as (p)ppGpp and sigma factors and by cis DNA sequences. The hypothesis underlying this project is that expression of bmp and other *B. burgdorferi* genes is modulated in trans by the stringent response both directly by (p)ppGpp and indirectly through sigma 54 and sigma S and in cis by the DNA sequences of their 5' upstream promoter regions. The specific aims of this project are: 1) characterize the regulation of rel/Bbu expression and the Rel/Bbu-mediated stringent response on bmp expression during growth of *B. burgdorferi* in BSKH media, in co-culture with tick cells, and in rat peritoneal culture chambers and ticks; 2) ascertain the roles of sigma S and sigma 54 in modulating bmp expression by the stringent response and their hierarchical interactions in *B. burgdorferi* in vitro and in vivo using bacterial genetics methods, DNA microarrays and proteomics; and 3) identify putative regulatory proteins binding to 5' upstream DNA sequences of the rpsL, bmpD, bmpC, bmpA and sigma S genes and their DNA binding sites. The proposed experiments will permit us to identify global factors, hierarchical regulatory networks and cis acting DNA sequences involved in the regulation of bmp genes, and will take studies of gene expression and modulation in *B. burgdorferi* from a descriptive paradigm to a paradigm framed by mechanistic and causal interpretations.

Grant: 2R01AI043197-06
Program Director: ZOU, LANLING
Principal Investigator: BOYAKA, PROSPER N PHD
Title: MOLECULAR ADJUVANTS FOR NALT-BASED IMMUNITY TO ANTHRAX
Institution: UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL
BIRMINGHAM
Project Period: 1998/03/15-2008/03/31

DESCRIPTION (provided by applicant): There is a major need to construct safe molecular adjuvants in new mucosal vaccine development, including those designed to protect from Category A. pathogens and exotoxins. Previous work on this grant focused on defining murine nasal-associated lymphoreticular tissues (NALT) as inductive sites by use of novel nontoxic derivatives of the classical mucosal adjuvant cholera toxin (CT). In addition, we have assessed regulatory cytokines and chemokines in these studies to characterize NALT-based mucosal immunity. These studies have also included characterization of human NALT, e.g., the tonsils and adenoids for both HIV infection as well as for optimal development of mucosal and systemic immunity to prevent sexual transmission of HIV. A total of five original Specific Aims were successfully addressed. The events of September 11, 2001 and the aftermath involving anthrax-tainted letters has led to important new initiatives to assess mucosal immunity to *Bacillus anthracis* and its three part exotoxin. For this reason, we have chosen to study murine NALT-based mucosal immunity to anthrax using the ADP-ribosylation deficient, molecular mutants of CT and chimeras consisting of A subunit of CT and B subunits of *Escherichia coli* labile toxin (LT). In this renewal grant, we have proposed five Specific Aims, which when completed, will provide essential new information regarding induction of and the functions for mucosal secretory IgA (S-IgA) and serum IgG subclass and IgA antibodies (Abs) in protection from anthrax, both in vitro and in vivo. The first Specific Aim will compare nontoxic mCTs with native CT given with protective antigen (PA) for induction of protective S-IgA Abs. The second Specific Aim will characterize a new panel of monoclonal anti-PA Abs (mAbs) which include all four IgG subclasses, IgE and IgA. The third Specific Aim will employ a newly developed *Brevibacillus* expression system to produce mCTs and mCT-A / LT-B chimera adjuvants and PA fusion protein as a potential nasal vaccine to protect from inhalational anthrax. The fourth Specific Aim will establish in vitro respiratory epithelial cells with sensitivity to the exotoxin of anthrax for in vitro studies of S-IgA anti-PA mAbs for neutralization of anthrax exotoxin. The last Specific Aim will establish an in vivo model of nasal administration of anthrax components for development of a method to assess nasal anthrax toxicity and mucosal immunity.

Grant: 2R01AI043312-04
Program Director: PERDUE, SAMUEL S.
Principal Investigator: JACOBY, GEORGE A MD
Title: Plasmid-Mediated Quinolone Resistance
Institution: LAHEY CLINIC BURLINGTON, MA
Project Period: 1999/03/15-2008/03/31

DESCRIPTION (provided by applicant): Quinolones are widely used antimicrobial agents because of their broad antibacterial spectrum, low toxicity, and reliable action against otherwise resistant pathogens. Bacterial resistance to quinolones, however, is increasing and has reached alarming levels in some parts of Europe and the Far East. Various chromosomal mutations contribute to this resistance. Plasmid-mediated resistance was long thought not to exist. We discovered a plasmid-encoded protein termed Qnr that protects DNA gyrase from quinolone inhibition. Qnr acts additively with chromosomal mechanisms for quinolone resistance, belongs to the pentapeptide repeat family of proteins and, by a gel displacement assay, binds to the gyrase tetramer as well as to the GyrA and GyrB subunits with differing affinities. Although initially found in a limited number of isolates in the United States, the qnr gene has recently been discovered in a larger group of isolates from the Far East. The aims of this proposal are: to elucidate the structural requirements for Qnr binding to gyrase, to establish the gyrase activities that Qnr can protect from quinolone inhibition, to determine whether Qnr can also protect the related quinolone target topoisomerase IV, to explore the hypothesis that Qnr blocks quinolone binding to gyrase, to study Qnr binding quantitatively using surface plasmon resonance, to investigate whether Qnr can also protect gyrase from such protein inhibitors as MccB17, CcdB, and Gyrl, to determine whether Qnr and an active gyrase fragment can co-crystallize for structural analysis by x-ray diffraction, and to study the detailed molecular structure of Qnr plasmids to elucidate how this novel property has been acquired. Qnr prevalence will also be explored utilizing samples from parts of the world where quinolone resistance has become common and also from food animal sources. These studies are important not only for understanding an emerging resistance mechanism but should also reveal details of how DNA gyrase and related topoisomerases function and how proteins in the pentapeptide family interact with and regulate the activity of other proteins.

Grant: 2R01AI043321-06
Program Director: BAKER, PHILLIP J.
Principal Investigator: DIETRICH, WILLIAM F
Title: Genetic Analysis of Lethal Factor Sensitivity
Institution: HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA
Project Period: 1998/06/01-2008/05/31

DESCRIPTION (provided by applicant): Anthrax lethal toxin (LeTx) is an important component of the pathogenesis caused by *Bacillus anthracis* infections. We have recently identified mutations in a kinesin gene (Kif1 C) that increase susceptibility of mouse macrophages to the cytotoxic effects of LeTx. While it is well known that Kif1 C is a motor protein that mediates the intracellular transport of vesicles, the exact functions of Kif1 C in cells under normal and LeTx-intoxicated conditions are not well described. Accordingly, in the first 2 Aims, we propose experiments that exploit the different alleles of Kif1 C to try to unravel important aspects of its function. Specifically, we will test if the susceptibility-inducing mutations affect Kif1 C protein abundance, the susceptibility of Kif1 C to LF mediated proteolysis, the intracellular distribution or phosphorylation of Kif1 C, and the interactions of Kif1 C with likely cargo vesicles and proteins. The third Aim is designed to explore some essential unexplained questions about the functioning of the anthrax toxins during anthrax pathogenesis. We propose to investigate the possibility of an interaction between edema toxin and lethal toxin in the cytolysis of macrophages. We also propose to search for human macrophage variation in LeTx susceptibility as a means to better understand the pathogenesis of human anthrax and perhaps provide an explanation of variation in human susceptibility to anthrax.

Grant: 2R01AI043346-06
Program Director: DUNCAN, RORY A.
Principal Investigator: KWON, GLEN S PHD
Title: Artificial Polymeric Lipoproteins as Drug Carriers
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 1998/07/01-2006/12/31

DESCRIPTION (provided by applicant): The clinical role of many drugs currently used to fight opportunistic infections (OIs) and the impact of many potent drugs for OIs coming out of massive drug discovery programs have been hampered by poor watersolubility, high toxicity, and inadequate parenteral dosage forms despite encouraging results in preclinical and clinical testing. Current efforts to address these major bottlenecks in drug development fall in the realm of nanotechnology. In particular, polymeric micelles, nanoscopic supramolecular core-shell structures, have recently entered clinical trials for potent yet poorly water-soluble and toxic drugs, owing to safety, high drug loading, and improved pharmacokinetics. A unique aspect of polymeric micelles is the ability to adjust their chemical structures to fine-tune properties for drug delivery. Our results suggest that adjustments must be made with an individual drug or class of drugs in mind, and that easily made adjustments on poly(ethylene oxide)-block-poly(L-amino acid) (PEG-b-PLAA) micelles may enhance drug delivery. Our efforts focus on amphotericin B (AmB), the primary drug for opportunistic systemic fungal infections. These OIs are a major cause of morbidity among immunocompromised patients suffering from cancer or AIDS and organ transplant recipients. We believe that tailor-made PEG-b-PLAA micelles may increase the therapeutic index of AmB. Specifically, we hypothesize that beneficial changes in the pharmacokinetics of AmB, increased plasma half-life and reduced liver clearance, and changes in its self-aggregation state, owing to PEG-b-PLAA micelles may lower the drug's toxicity and increase its antifungal efficacy. In this context, we may adjust the structure of PEG-b-PLAA micelles to fine-tune the release kinetics of AmB and enhance its delivery. Specific Aims: (1) To study the pharmacokinetics (plasma profile, distribution in plasma, and tissue distribution) of AmB encapsulated by PEG-b-PLAA micelles in rodents. (2) To study the acute, renal and liver toxicity of AmB encapsulated in PEG-b-PLAA micelles in rodents. (3) To study the antifungal activity of AmB encapsulated in PEG-b-PLAA micelles in a neutropenic murine model of disseminated candidiasis. Comparisons will be made with a standard formulation of AmB and a liposomal AmB approved for refractory systemic fungal diseases. These proposed studies will provide insight into mechanisms behind the toxicity and antifungal activity of AmB and perhaps show that PEG-b-PLAA micelles increase the therapeutic index for the drug.

Grant: 2R01AI043559-04A1
Program Director: SCHMITT, CLARE K.
Principal Investigator: GUERRY, PATRICIA
Title: Glycosylation of Campylobacter flagella
Institution: HENRY M. JACKSON FDN FOR THE ADV MIL/MED ROCKVILLE, MD
Project Period: 1999/07/01-2008/03/31

DESCRIPTION (provided by applicant): Campylobacter jejuni is the leading cause of foodborne illness in North America and is among the major causes of bacterial diarrhea worldwide. Flagella and motility are required for intestinal colonization and invasion of intestinal epithelial cells by C. jejuni, and flagellin is an immunodominant and possibly a protective antigen. Flagellin from C. jejuni strain 81-176 and Campylobacter coli strain VC167 are glycosylated at 19 and 16 serine or threonine residues, respectively, with a 9 carbon sugar called pseudaminic acid and derivatives of pseudaminic acid. The modifications, which account for approximately 10% of the weight of these glycoproteins, are surface exposed on the flagella filament and are likely involved in interaction of flagellin with the eukaryotic host. Genetic analyses indicate that the pathway for biosynthesis of pseudaminic acid is conserved in both 81-176 and VC167. Flagellins from both strains contain minor modifications that are acetamidino forms of pseudaminic acid (mass 315 Da). However, the 315 Da group synthesized by 81-176 and VC 167 are structurally and immunologically distinct and are synthesized by independent pathways in each organism. The data suggest that campylobacter flagellin needs to be glycosylated in order to be exported and/or assembled into a filament. A mutant in 81-176 that is unable to synthesize the acetamidino form of pseudaminic acid appears to be attenuated in virulence. The aim of this study is to further elucidate the pathways by which the different forms of pseudaminic acid are synthesized and to study unique aspects of the regulation of these glycosylation genes. Site-specific mutagenesis will be done on flagellin to eliminate modification sites sequentially in order to determine sites that are critical for flagella function and the rules of site occupancy. The biological role of flagella glycosylation will be studied by examining a series of mutants in in vitro and in vivo assays of virulence.

Grant: 2R01AI043846-06
Program Director: NEAR, KAREN A.
Principal Investigator: BISHAI, WILLIAM R MD
Title: Toolbox for Tuberculosis Drug Development
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 1998/08/01-2007/12/31

DESCRIPTION (provided by applicant): HIV combined with either latent or active tuberculosis poses devastating consequences both in the U.S. and overseas. With no new drug class approved for tuberculosis since 1967 and with the growing problem of multidrug-resistant tuberculosis (MDRTB), the development of new agents to treat active or latent TB in the setting of HIV is a high priority. Modern drug discovery is a pipeline, which initiates with the identification of an appropriate target and culminates in pre-clinical evaluation of drug candidates and clinical trials. In this application we propose basic research on the identification of drug targets plus the development of several novel approaches to preclinical antimicrobial characterization in vitro and in vivo. A central tenet of the first aim of the application is that the best drug targets are those which are strictly essential for the organism. Through the use of random transposon mutagenesis, precise characterization of 1425 mutants by DNA sequencing, and a Monte Carlo biostatistical model of essential genes, we have identified 878 non-essential genes of *M. tuberculosis* and 22 genes with probabilities of being essential ranging from 93-75%. Several of the likely essential genes have known inhibitors which we have found to be active against *M. tuberculosis* in vitro. We propose to extend this work to identify and characterize additional essential gene targets. Secondly towards advancing the later-stages of the drug development pipeline, we have developed the use of diffusion bioreactors with flow rate control for assessing critical pharmacodynamic parameters of drugs active against mycobacteria. In a related vein we have developed traditional and novel animal models which may predict drug activity against human latent tuberculosis infection (LTBI). We propose to extend the use of these tools, and to evaluate novel preventive therapy regimens for LTBI in the setting of exposure to drug-resistant TB such as might occur in areas with high MDRTB prevalence or in a bioterrorism attack.

Grant: 2R01AI044170-05
Program Director: VAN DE VERG, LILLIAN L.
Principal Investigator: BAUMLER, ANDREAS PHD
Title: Bovine Specific Virulence Factors of S.typhimurium
Institution: TEXAS A&M UNIVERSITY HEALTH SCIENCE COLLEGE STATION, TX
CTR
Project Period: 1998/12/01-2007/05/31

DESCRIPTION (provided by applicant): Salmonella serotypes are the leading cause of food-borne infections with lethal outcome in the United States. The pathogenesis of this diarrheal disease has not been intensively studied in Salmonella since diarrhea does not develop in rodent models. In contrast, calves infected with S. Typhimurium develop similar signs of disease and pathology as observed in humans. Our long-range goal is to elucidate the molecular mechanisms involved in the pathogenesis of S. Typhimurium-induced enterocolitis. The objectives of this application are to study how the invasion associated type III secretion system (TTSS-1) of S. Typhimurium directs migration of neutrophils into the intestinal mucosa and to determine the role of neutrophils in causing fluid accumulation and intestinal pathology in the calf. Our central hypothesis is that upregulation of CXC chemokines in the bovine intestinal mucosa induced by TTSS-1 secreted effector proteins elicits a neutrophil influx, which is the main mechanism leading to tissue injury and diarrhea during S. Typhimurium-induced enterocolitis. The rationale for the proposed research is that a better understanding of the complex series of events leading to inflammation and fluid accumulation during the interaction of S. Typhimurium with host tissue in vivo will be required for the development of new and innovative approaches for treatment and prevention. We will test different aspects of our hypothesis by pursuing the following four specific aims: (1) Determine the role of TTSS-1 in inducing the production of CXC chemokines in the intestinal mucosa; (2) Investigate CXC chemokine expression in bovine tissue on the cellular level; (3) Determine the contribution of CXC chemokines to neutrophil recruitment during S. Typhimurium induced enterocolitis; (4) Determine the role of neutrophils in S. Typhimurium induced fluid accumulation. We are particularly well prepared to perform the proposed studies because we have established the calf model of human enterocolitis and have identified S. Typhimurium virulence factors, which are essential for causing this disease syndrome. It is our expectation that our approach will establish the complex series of events leading to fluid accumulation during enterocolitis. This outcome will be significant because identification of the key events during S Typhimurium interaction with host tissue in vivo will have a considerable impact on the design of in vitro models used by other investigators in the field.

Grant: 2R01AI044424-04A1
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: STACZEK, JOHN
Title: Chimeric Virus Vaccines for *P. aeruginosa* Infections
Institution: LOUISIANA STATE UNIV HSC SHREVEPORT SHREVEPORT, LA
Project Period: 1998/07/01-2007/03/31

DESCRIPTION (provided by applicant): *Pseudomonas* infection is an underappreciated cause of morbidity and mortality. Nosocomial infections can be life-threatening in immunocompromized populations, cancer patients, the elderly, and patients with cystic fibrosis. Physicians try to protect patients with antibiotic therapy, but the bacteria quickly develop antibiotic resistance. A complementary approach to antibiotic therapy is therefore urgently needed, and one such approach is vaccination. Our long-range goal is to develop vaccines that protect against *Pseudomonas* lung infection. We have developed two effective outer membrane protein F (OprF)-based vaccines that protect against both nonmucoid and mucoid *Pseudomonas* phenotypes. These vaccines are called F/I and F/HG. The F/I vaccine consists of three biolistic inoculations of naked DNA sequences for the fusion protein OprF/I. The F/HG vaccine uses a prime-boost strategy with two biolistic inoculations of naked DNA-oprF sequences followed by an intramuscular booster containing the chimeric influenza virus HG10-11. Each vaccine appears to induce a polarized immune response. The F/I vaccine induced antibody-mediated immunity (AMI) while F/HG induced cell-mediated immunity (CMI). Insufficient information is available regarding the immune mechanisms whereby *Pseudomonas* infection is controlled or how *Pseudomonas* vaccines work. AMI in pulmonary *Pseudomonas* infection is believed to be important, but the definitive mechanism for clearance is unknown. We propose to define the mechanisms of antibody protection by identifying antibody isotypes and serum cytokines in infected and F/I-immunized mice that are immune-intact or immune-deficient. Likewise, the role of CMI in *Pseudomonas* pneumonia is poorly understood. Our F/HG vaccine will allow us to define the mechanism(s) of *Pseudomonas*-specific, cell-mediated protection in the lungs of infected and immunized mice that are immune-intact or immune-deficient. Defining these mechanisms will allow us to rationally modify immune responses to protect more effectively against pulmonary *Pseudomonas* infection. As researchers delineate the immune responses to pulmonary *Pseudomonas* infection in humans, we will be uniquely positioned to modify our vaccines to induce specific Th-1 or Th-2 responses. These rationally designed vaccines tested in a pulmonary chronic infection model will provide guiding principles to prevent and treat more effectively *Pseudomonas* pneumonia in humans.

Grant: 2R01AI045014-05
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: RAMPHAL, REUBEN
Title: PSEUDOMONAS AERUGINOSA FLAGELLAR BIOGENESIS PATHWAY
Institution: UNIVERSITY OF FLORIDA GAINESVILLE, FL
Project Period: 1999/02/01-2008/02/28

DESCRIPTION (provided by applicant): The flagellum expressed by pathogenic microorganisms functions at several stages of infection. In addition to their role in motility, some flagella are attachment organelles, others are used for the secretion of virulence factors, and all are likely to be potent stimuli in triggering the innate immune response and inflammation. The single flagellum of *P. aeruginosa* serves some of these functions in disease and plays a pivotal role in the formation of bacterial biofilms in nature and possibly in lungs. The long-term goal of this proposal is therefore to understand the biogenesis of this organelle in nature and disease. The major regulatory steps in the flagellar biogenesis have been elucidated but little is known about its regulation in disease. Recent findings demonstrate that growth of *P. aeruginosa* in respiratory mucus results in a block in flagellin synthesis. The purpose of repression of flagellin synthesis may be to avoid production of potent innate immune response activators and perhaps prevent opsonophagocytosis. The hypothesis that derives from this observation is that down-regulation of flagellin may be a prerequisite for chronic colonization of mucus in Cystic Fibrosis. The specific aims of this proposal are therefore: 1. Identify the regulatory network which controls flagellin expression and hence flagellum assembly in mucus. 2. Identify the signal for flagellin down-regulation in mucus. 3. Engineer strains of *P. aeruginosa* that constitutively express a flagellum in mucus and assess their virulence in animal models of infection. The regulatory network that directs the repression of flagellin production within the normal biogenesis pathway will be ascertained by examining the role of an inhibitor of flagellin synthesis, FlgM, in this process if any. The action of novel regulatory mechanisms that may function independent of the flagellar biogenesis pathway will also be examined. The source of the signal in mucus, which controls flagellin expression, will be sought by examining whether adhesion to mucins, or another component of mucus provides these environmental signals. Lastly, *P. aeruginosa* strains lacking suppression of flagellin synthesis in mucus will be engineered and examined in both an acute and a chronic model of *P. aeruginosa* lung infection. If flagellin synthesis is deleterious to chronic colonization, this may provide an opportunity for therapeutic intervention, where ectopic expression of flagellin in mucus may be driven to allow host defenses to deal with the organism early in the colonization process.

Grant: 2R01AI045580-05
Program Director: PERDUE, SAMUEL S.
Principal Investigator: BARBET, ANTHONY F PHD OTHER AREAS
Title: Molecular Mechanisms of msp2 Variation in Rickettsiae
Institution: UNIVERSITY OF FLORIDA GAINSVILLE, FL
Project Period: 1999/07/01-2007/12/31

DESCRIPTION (provided by applicant): The objective of this proposal is to improve our understanding of the mechanisms of pathogenesis of tick borne rickettsial pathogens that cause ehrlichiosis and anaplasmosis of humans and animals. These pathogens efficiently utilize a small genome (<1.5 Mb) to evade the immune response and establish persistent infection in the mammalian reservoir host, to colonize and replicate in the tick midgut and salivary glands, and to develop infectivity upon renewed feeding of the tick to effect onward transmission. MSP2 was initially defined in *Anaplasma marginale* and infections of cattle and ticks with this pathogen provide an excellent model for discovering the mechanisms used to modify the surface proteome. In the prior project period, we identified segmental gene conversion of single expression sites for MSP2, and a related surface paralogue MSP3, as a primary mechanism for generating surface diversity and demonstrated differential expression of operon-encoded proteins between the mammalian host and tick vector. A similar gene conversion mechanism is used by *Anaplasma phagocytophilum* to express a large repertoire of outer membrane proteins in human patients and studies by others support expression from multiple loci to generate surface diversity. Analysis of the *A. marginale* genome reveals a complex family of outer membrane protein genes related to msp2. This msp2 superfamily is comprised of 32 paralogues, comprising the two msp2 and msp3 operon-linked expression sites, a single msp4 gene locus, multiple msp2 and msp3 pseudogenes, and other uncharacterized msp2-like paralogues. We hypothesize that differential expression of these paralogues and recombination between them generates diversity in the pathogen surface and provides the ability of organisms to adapt to and persist in different hosts and cellular environments. The specific aims of the present proposal are: 1] Determine if msp2 superfamily genes are differentially expressed during infection of the mammalian and invertebrate hosts; 2] Determine the operon structure and generation of diversity within msp2 superfamily gene clusters; 3] Identify the mechanisms for differential regulation of the msp2 superfamily proteins in the mammalian and invertebrate hosts; and 4] Compare regulation of expression of msp2 superfamily proteins in *A. marginale* and *A. phagocytophilum*.

Grant: 1R01AI048622-01A2
Program Director: VAN DE VERG, LILLIAN L.
Principal Investigator: LIBBY, STEPHEN J PHD
Title: The Sly A Regulon in Salmonella Pathogenesis
Institution: NORTH CAROLINA STATE UNIVERSITY RALEIGH, NC
RALEIGH
Project Period: 2003/02/15-2003/09/30

DESCRIPTION (provided by applicant): Salmonella infections continue to pose a significant threat to human health worldwide. Our studies have established an essential role for the slyA gene in the pathogenesis of Salmonella infections. The SlyA protein belongs to a novel family of low molecular weight transcriptional regulators. SlyA appears to be maximally expressed in stationary phase cultures and in the intracellular environment of phagocytes, slyA mutant Salmonella typhimurium is profoundly attenuated for virulence in a murine model of salmonellosis, unable to survive and replicate within phagocytes, and hypersusceptible to oxidative stress. By DNA microarray analysis, we have identified a number of candidate SlyA-regulated genes. To determine the mechanism by which the SlyA regulon defends S. typhimurium against oxidative stress and contributes to Salmonella pathogenesis, the following Specific Aims of this revised proposal are: (1) Identification and characterization of SlyA-dependent genes. Preliminary experiments have successfully identified a number of candidate SlyA-dependent loci, which will be confirmed by several independent methods (mRNA, protein, reporter fusions). A SlyA-regulated gene in Salmonella Pathogenicity Island-4 designated STM4261 that encodes a large protein with a serine protease motif will be biochemically characterized. STM4261 expression will be measured in wild type and slyA mutant backgrounds, and the virulence of non-polar mutants of STM4261 will be determined. (2) Definition of the role of SlyA-dependent genes in oxidative stress resistance and virulence. The contribution of individual SlyA-dependent loci to oxidative stress resistance, growth in phagocytes, and S. typhimurium virulence will be determined. (3) Molecular analysis of slyA regulation. Transcriptional and translational mechanisms governing slyA expression in S. typhimurium will be determined. Regulatory interactions between SlyA and PhoPQ will be explored. A novel two-component regulatory locus that appears to be essential for slyA expression will be characterized, slyA-dependent promoters will be analyzed, and a consensus binding sequence will be determined. The overall goal of this project is to understand mechanisms by which the SlyA regulon confers resistance to the oxidative stress encountered by Salmonella in host phagocytes. The slyA gene family is conserved among Gram-negative enteric pathogens, as well as several important plant pathogens. Understanding the molecular mechanisms by which the SlyA regulon functions in Salmonella promises to reveal novel mechanisms for intracellular survival of pathogenic bacteria as well as provide important general insights into the evolutionary adaptation of bacteria to oxygen-rich environments.

Grant: 1R01AI049322-01A2
Program Director: KLEIN, DAVID L
Principal Investigator: ST GEME, JOSEPH W MD
Title: Haemophilus Hap-mediated Microcolony Formation
Institution: WASHINGTON UNIVERSITY ST LOUIS, MO
Project Period: 2003/01/01-2007/12/31

DESCRIPTION (provided by applicant): Nontypable *Haemophilus influenzae* is a common cause of localized respiratory tract disease, including otitis media, sinusitis, bronchitis, and pneumonia. In addition, this organism causes serious systemic disease, such as meningitis, endocarditis, and septicemia. The initial step in the pathogenesis of nontypable *H. influenzae* disease involves colonization of the upper respiratory mucosa. We have identified an *H. influenzae* serine protease called Hap, which facilitates intimate interaction with epithelial cells and extracellular matrix proteins and also promotes bacterial aggregation and microcolony formation. Based on our in vitro results, we speculate that Hap plays an important role in the process of colonization. Hap belongs to the growing family of autotransporter proteins and is synthesized as a precursor protein with 3 functional domains, including an N-terminal signal sequence, an internal protease domain with adhesive activity (Haps), and a C-terminal outer membrane domain with translocator activity (HapBeta). Ultimately, Hap undergoes autoproteolytic cleavage, with extracellular release of Haps. In recent work, we demonstrated that Hap mediated adherence and microcolony formation are potentiated by a host protein called secretory leukocyte protease inhibitor (SLPI). This protein is present in respiratory secretions and inhibits Hap autoproteolysis, resulting in accumulation of surface-associated Haps. In the present proposal, we will focus on Hap-mediated adherence and microcolony formation. In Aim 1, we will solve the crystal structure of Haps and define the interactive surfaces involved in adherence and microcolony formation. In Aim 2, we will examine the ability of microcolonies to resist killing by cationic peptides, to evade macrophage phagocytosis, and to enhance persistence in the chinchilla otitis media model. In Aim 3, we will characterize the relationship between respiratory viral infection and Hap-mediated adherence and microcolony formation, concentrating on the role of SLPI. From a practical perspective, the proposed studies may facilitate efforts to develop novel strategies for the treatment and prevention of *H. influenzae* disease. Perhaps more importantly, they may provide general insights into host-microbe relationships and expand our understanding of microbial biofilms.

Grant:	1R01AI049417-01A2	
Program Director:	KORPELA, JUKKA K.	
Principal Investigator:	ROSEN, HENRY	MD OTHER CL MED:CLINICAL MEDICINE,UNSPEC
Title:	Microbial Response to Neutrophil Phagocytosis	
Institution:	UNIVERSITY OF WASHINGTON	SEATTLE, WA
Project Period:	2003/04/01-2007/03/31	

DESCRIPTION (provided by applicant): Neutrophils are a key component of host defense against bacterial and fungal pathogens. When neutrophils are severely diminished in number or function, the host becomes highly susceptible to serious infection. Neutrophils typically ingest and kill bacteria within a short space of time. In order to gain a better understanding of the bacterial response to phagocytosis, genomic arrays will be used to assess changes in mRNA abundance for *Escherichia coli* cells ingested by neutrophils. We expect to learn how ingested bacteria prioritize responses to stresses generated in the phagocyte and whether these responses enhance microbial survival. The effects of neutrophils deficient in antimicrobial systems mediated by the phagocyte NADPH oxidase (CGD, chronic granulomatous disease) or the enzyme myeloperoxidase will be compared with normal neutrophils. Initial studies indicate that normal neutrophils, but not CGD neutrophils, evoke an anti-oxidant response mediated by the bacterial transcription factor, OxyR. Disruption of the *oxyR* gene generated a bacterial strain that was 10-fold more susceptible to killing by normal neutrophils. The *oxyR* strain also appears to be markedly attenuated for virulence in a mouse model of ascending pyelonephritis. This proposal seeks to characterize *oxyR* effects more fully, and to explore the effects of selected other major transcriptional responses both to neutrophil phagocytosis and to isolated antimicrobial model systems. It also seeks to implement methodology that will emphasize detection of phagocytosis-induced changes for mRNA transcripts present in a pathogenic *E. coli* strain but not in a laboratory-adapted strain. The driving hypothesis for the latter goal being that: pathogen-specific transcripts contribute substantially to the pathogenic phenotype. Expression profiling of phagocytosed bacteria appears to provide useful information about conditions in the phagocytic vacuole and about important bacterial defenses mounted in response to this hostile environment.

Grant: 1R01AI050011-01A2
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: CLEGG, STEVEN PHD
Title: Pathogenesis of Klebsiella airway infections
Institution: UNIVERSITY OF IOWA IOWA CITY, IA
Project Period: 2003/04/01-2008/03/31

DESCRIPTION (provided by applicant): The opportunistic pathogen, *Klebsiella pneumoniae*, is responsible for a significant number of pulmonary infections in compromised individuals. The ubiquity of antibiotic resistant strains, particularly those producing extended-spectrum beta-lactamases, presents a serious clinical problem among groups such as hospitalized individuals and chronic alcoholics. The pathogenesis of *Klebsiella* airway infections has not been studied to any great extent and the investigation of the production of virulence determinants has essentially focused upon the role of capsules as antiphagocytic factors. The mouse has been extensively used as a model of airway infections due to *K. pneumoniae* primarily to investigate host cell responses. Also, epidemiologic observations suggest that specific capsular serotypes (e.g. K2) are most frequently associated with pulmonary infections. However, our preliminary data indicate that not all K2-positive isolates are virulent in the mouse model of infection. Therefore, although the capsule is most likely to be an antiphagocytic factor and prevent efficient killing of the bacteria in vivo, additional factors are necessary to establish airway infections with subsequent invasion of the bloodstream. We propose to identify and confirm the role of previously unknown virulence factors that mediate airway infections due to *K. pneumoniae*. Three techniques; signature-tagged mutagenesis, subtractive hybridization and in vivo gene expression technology will identify these determinants. The murine model of *Klebsiella* infection will be used to demonstrate the role of putative virulence factors during infection. The three approaches are complementary and have been used to investigate virulence in many different types of pathogens. Since very little is known about the virulence factors of *K. pneumoniae*, it is anticipated that these studies will provide information on new and novel virulence factors produced by these bacteria. Fundamental to devising new therapeutic approaches to opportunistic infections will be an understanding of the virulence factors produced by this group of organisms.

Grant: 1R01AI050038-01A2
Program Director: SAWYER, LEIGH A.
Principal Investigator: EISENBERG, JOSEPH N MS
Title: Environmental change and diarrheal disease
Institution: UNIVERSITY OF CALIFORNIA BERKELEY BERKELEY, CA
Project Period: 2003/01/15-2007/12/31

DESCRIPTION (provided by applicant): Diarrheal diseases are predictable outcomes when fecal-oral pathogens meet human poverty and-dislocation. Are diarrheal diseases sensitive indicators of change in socioeconomic level, resource availability, and human social contacts? Processes like these are extremely difficult to study in a controlled fashion. The construction of a new road in coastal Ecuador provides a valuable natural experiment for this purpose. This road will link some previously remote villages to local, regional, and national networks of goods, services, and people, creating new connections among them. We hypothesize that: (1) the level and type of connections between villages are correlated with infection of enteric pathogens; and (2) the changing social connections, new resources, and sanitary and hygienic behaviors of individuals within villages are correlated with infection of enteric pathogens. These hypotheses use the village as their unit of analysis. When estimating the contribution of various exposure risks to disease incidence, it is also important to explore the implications of interdependence between these pathways. This is the third aim of our study: analyzing the joint effects of changes in these contact patterns using disease transmission models. Using a quasi-experimental design, twenty villages will be followed for 4 years, selected so that a rural-urban continuum is fully represented. This continuum will be measured by several factors that relate a given village to Borbon, the town located at the confluence of two rivers that support the villages within the region. Data will be collected at three levels. First, health promoters who live in the villages under study will implement an active surveillance program. They will administer a new survey tool to measure the incidence of all the diarrheal illnesses and monitor both proximal and distal determinants of disease, in a given village. Second, a biannual visit to each community will be conducted by our field team, which has recently completed a cross sectional feasibility study in these villages. Each visit will last two weeks. In these visits, stool samples will be collected from all symptomatic individuals and a random selection of controls. In addition, during these visits, survey tools will be used to collect information on water-use behavior sanitation, hygiene, food consumption patterns, and travel and migration. Third, semi-annual visits to each village will be undertaken by the local Ecuadorian anthropologist. These visits will include open-ended interviews as well as additional questions about social network formation and change. The visits will also allow village development and road.

Grant: 1R01AI050107-01A2
Program Director: KORPELA, JUKKA K.
Principal Investigator: DAVID, SUNIL A MD
Title: Rational Development of Endotoxin Sequestering Agents
Institution: UNIVERSITY OF KANSAS LAWRENCE LAWRENCE, KS
Project Period: 2003/05/01-2007/04/30

DESCRIPTION (provided by applicant): Lipopolysaccharides (LPS) or endotoxins are outer membrane constituents of gram-negative bacteria that play a key role in the pathogenesis of septic shock, a leading cause of mortality worldwide for which there is as yet no effective therapy. One possible approach to developing novel therapeutic strategies to treat sepsis is to sequester circulating LPS, a strategy that has been historically addressed using monoclonal antibodies directed against the structurally conserved lipid regions of LPS. However, a series of clinical trials using monoclonal antibodies have been unsuccessful owing to the lack of accessible recognition sites on the lipid. Our previous work on identifying structural requisites necessary for binding and neutralization of LPS in a variety of proteins, peptides and small molecules led to the identification of a novel class of structurally simple, nontoxic molecules, the lipopolyamines, which bind and neutralize LPS in vitro, and afford protection against LPS challenge in two murine models of gram-negative sepsis. Embodying an interdisciplinary approach, we propose to synthesize several homologous series of novel compounds rationally designed to maximize binding affinity and neutralization potency, and to exhibit desirable pharmacokinetic and toxicological profiles, based on optimal structural templates that we have already established with the lipopolyamines. Employing a hierarchical screening strategy, the interactions of these molecules with LPS will be comprehensively evaluated employing a variety of biophysical methods, including the determination of dissociation constants. Test compounds will be screened for the ability to inhibit LPS-induced cellular activation and production of key proinflammatory mediators of septic shock. Highly active molecules will be further tested in two murine models of gram-negative sepsis. The toxicity of the compounds will be systematically determined in a panel of in vitro assays.

Grant: 1R01AI050652-01A1
Program Director: SCHMITT, CLARE K.
Principal Investigator: CRANE, JOHN K
Title: Host Cell Killing by EPEC: Central Role in Pathogenesis
Institution: STATE UNIVERSITY OF NEW YORK AT AMHERST, NY
BUFFALO
Project Period: 2003/09/30-2006/01/31

DESCRIPTION (provided by applicant): Enteropathogenic *E. coli* (EPEC) is a common cause of severe, watery diarrhea in children in developing countries. EPEC is also the prototype of a group of attaching and effacing intestinal pathogens, including enterohemorrhagic *E. coli* (EHEC, such as O157:H7), *Citrobacter rodentium*, *Hafnia alvei*, and EPEC-like *E. coli* strains of domestic animals. Unlike many other *E. coli* strains that cause diarrhea, EPEC produces no known toxins, so the way it causes disease has been puzzling. Despite major advances in understanding how EPEC adhere, trigger cytoskeletal rearrangements in the host, and cause other host cell alterations, the mechanism by which EPEC causes diarrhea has been unclear. The discovery that EPEC triggers host cell death provided an important lead in how EPEC causes disease. The mode of cell death triggered by EPEC has features of both apoptosis (programmed cell death) and necrosis. One of the non-apoptotic features of EPEC-mediated killing is release of adenosine triphosphate (ATP) from the host cell. Once released, ATP is broken down to other adenine nucleotides and adenosine. Adenosine itself acts as a potent secretagogue, i.e., a stimulator of intestinal fluid and electrolyte secretion, which may cause or contribute to watery diarrhea. The present application seeks to understand how EPEC triggers the ATP release from the host, with a particular focus on the role of the cystic fibrosis transmembrane regulator (CFTR). Other goals include determining the signaling pathways activated by adenosine which activate intestinal secretion, and the determining the extent of release of adenine nucleotides into the intestinal tract of rabbits infected with the EPEC-like pathogens rabbit diarrheagenic *E. coli* (RDEC-1) and rabbit EPEC (REPEC).

Grant: 1R01AI050660-01A1
Program Director: VAN DE VERG, LILLIAN L.
Principal Investigator: FANG, FERRIC C MD CLINICAL MEDICAL SCIENCES, OTHER
Title: DNA DAMAGE AND REPAIR IN SALMONELLA PATHOGENESIS
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2003/04/01-2008/03/31

DESCRIPTION (provided by applicant): Phagocytic cells inhibit microbes through production of genotoxic reactive oxygen and nitrogen species (ROS/RNS) produced by the NADPH phagocyte oxidase (phox) and inducible nitric oxide synthase (iNOS). *Salmonella typhimurium* must repair DNA damage to resist killing by phagocyte-derived ROS/RNS and cause lethal infection in mice. The specific aims of this project are to: A) Determine effects of ROS/RNS on DNA repair-deficient *Salmonella* in vitro; B) Characterize DNA damage and essential DNA repair mechanisms during *Salmonella* infection in vivo; C) Identify specific DNA-binding zinc metalloproteins targeted by RNS/ROS. Preliminary observations suggest the hypothesis that inhibition of DNA replication is the final common pathway of DNA damage during infection. Replication arrest can be caused by multiple mechanisms including blocking lesions, strand breaks, nucleotide depletion or inhibition of the primosome apparatus required to restart collapsed replication forks. In the absence of the RecBC repair proteins, replication arrest can result in lethal double-strand breaks. Mobilization of zinc by RNS strongly correlates with cytostasis in vitro, suggesting that RNS inhibit DNA replication by targeting DNA-binding zinc metalloproteins. To test the central hypothesis of this proposal, strains of *S. typhimurium* deficient in excision repair, homologous recombination, or translation DNA synthesis will be constructed and examined for susceptibility to ROS/RNS. Measurement of DNA synthesis, strand breaks, and mutagenesis will clarify mechanisms of ROS/RNS-mediated DNA damage. Wild-type and congenic phox/iNOS knock-out macrophages and mice will be used to identify repair mechanisms required for *Salmonella* virulence and characterize host-derived mediators responsible for DNA damage and replication arrest during host-pathogen interactions in vivo. Biochemical strategies and site-specific mutagenesis will be utilized to identify zinc metalloproteins modified by RNS. These studies will provide novel insights into mechanisms by which innate host defenses limit microbial replication by targeting DNA synthesis and establish critical mechanisms of microbial resistance to ROS/RNS-related DNA damage.

Grant: 1R01AI050675-01A1
Program Director: KORPELA, JUKKA K.
Principal Investigator: BADGER, JULIE L PHD
Title: Role of TraJ in Neonatal E. coli Sepsis and Meningitis
Institution: CHILDREN'S HOSPITAL LOS ANGELES LOS ANGELES, CA
Project Period: 2003/04/01-2008/03/31

DESCRIPTION (provided by applicant): Despite the use of modern therapeutics and diagnostic measures, neonatal bacterial sepsis and meningitis continues to be a disease with unacceptable rates of morbidity and mortality. The fatality rate of this disease is 15-75 percent; furthermore, approximately 50 percent of meningitis survivors have significant neurological and developmental abnormalities. The primary, route of infection for E. coli K1 (the most common causative agent of gram-negative meningitis) is oral. Following intestinal colonization, bacteria translocate through the GI tract to extra-intestinal sites of mesenteric lymph nodes (MLN), liver, spleen, and the blood. E. coli K1 then multiply systemically within the bloodstream reaching a necessary threshold of bacteremia to gain access to the central nervous system (CNS). Recently we identified an E. coli K1 plasmid-encoded blood-brain barrier (BBB) invasion gene, *traJ*, with homology to *traJ* of various F-like plasmid *tra* operons. Our preliminary data indicate that the *traJ* mutation specifically attenuates dissemination from the MLN to the liver, spleen, blood, and the CNS in the neonatal rat. In addition, although animals orally infected with the *traJ* mutant demonstrated a decrease or no recoverable bacteria in the liver or spleen, these tissues showed a significant inflammatory response. In vitro studies determined that the *traJ* mutant is taken-up less by macrophages and shows a loss of a 55 kDa-secreted protein. The central hypothesis of this application is that TraJ controls the expression of a set of genes whose products (i.e., 55 kDa secreted protein) are involved in E. coli K1 dissemination, systemic infection and crossing the BBB, and these events within the disease process occur via TraJ-dependent interactions with professional phagocytes. The following proposed experiments are designed to test and substantiate our hypotheses. We aim to 1) Elucidate the genetic and molecular characteristics of the *traJ* region and plasmid, evaluate the ability of the endogenous plasmid to self-transfer, and determine the potential role of the *traJ*-containing plasmid in E. coli K1 neonatal rat virulence, 2) Evaluate the function of the TraJ-regulated proteins (i.e., 55 kDa-secreted protein) in E. coli K1 systemic dissemination and meningitis, and 3) Determine the mechanism of TraJ-dependent host inflammatory response in the neonatal rat. Improved knowledge of molecular mechanisms for early systemic dissemination and the exact interplay of the host inflammatory response during these events will assist in achieving our long-term goal of identifying novel rational approaches to development of new treatments and preventive strategies for E. coli K1 sepsis and meningitis.

Grant: 1R01AI050733-01A2
Program Director: SCHMITT, CLARE K.
Principal Investigator: BOUTON, AMY H PHD
Title: Mechanistic aspects of integrin-mediated Yersinia uptake
Institution: UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA
CHARLOTTESVILLE
Project Period: 2003/04/01-2008/03/31

DESCRIPTION (provided by applicant): Microbial pathogenicity is largely determined by virulence factors that interface with host cells and tissues to promote colonization and survival. The goal of this proposal is to develop a better understanding of the mechanisms through which virulence factors expressed by enteropathogenic Yersiniae utilize host cell factors to promote disease. One of the major virulence factors produced by these bacteria is invasin, which plays a crucial role in pathogenicity by binding to beta-1 integrins present on the surface of host cells and facilitating invasion of the intestinal epithelium. This proposal tests the hypothesis that specific integrin dependent signaling networks in the host are targeted by the Yersiniae to promote invasion. Whereas these pathways are activated during the initial invasion process, however, they are effectively subverted at later stages in infection by the activities of other virulence factors, thus allowing for extracellular growth of the pathogen. A second facet of this hypothesis is that components of integrin-dependent signaling networks eventually overcome the inhibitory actions of these virulence factors and facilitate clearance of the bacteria from the intestine. The following specific aims have been developed to address these hypotheses: Aim 1: Using in vitro cell culture models, we propose to investigate the molecular organization and regulation of integrin-dependent signaling networks established in macrophages during the course of Yersinia uptake. Aim 2: One component of integrin-dependent signaling pathways that has been strongly implicated in aspects of Yersinia pathogenicity is the Src family kinases. This aim will explore the function of these proteins in the course of Yersinia infection with the use of Src family knockout mice. Aim 3: These mouse models will also be used, together with a panel of Yersinia strains that contain mutations in specific cellular adhesins, to explore the functional relationship between Yersinia virulence factors and Src family kinases during the course of bacterial invasion and colonization. Completion of these studies will help to generate a comprehensive understanding of the host cell and microbial mechanisms involved in Yersinia pathogenicity and, in the process, provide insight into other integrin-dependent pathways.

Grant: 1R01AI050875-01A2
Program Director: PERDUE, SAMUEL S.
Principal Investigator: HAMBLIN, MICHAEL R PHD
Title: Photodynamic Therapy of Localized Infections
Institution: MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA
Project Period: 2003/07/01-2006/12/31

DESCRIPTION (provided by applicant): The overall goal of this proposal is to explore a novel photochemical method for killing antibiotic resistant pathogenic bacteria in localized models of infection. Photodynamic therapy (PDT) employs a non-toxic dye termed a photosensitizer (PS) and low intensity visible light, which in the presence of oxygen produce cytotoxic species. PDT has the advantage of dual selectivity in that the PS can be targeted to its destination cell or tissue, and in addition the illumination can be spatially directed to the lesion. PDT has previously been used to kill pathogenic microorganisms in vitro, but until now this has not been accomplished in animal models of infection. We have developed a novel method of targeting PS conjugates to both Gram (+) and Gram (-) pathogenic bacteria that can produce up to 6 logs of killing in vitro, while in vivo it increases the selectivity of the treatment for bacteria while sparing host tissue. This is based on the covalent attachment of the PS chlorin e6 to polycationic delivery vehicles such as poly-L-lysine, that increases the selective binding to bacteria and enables the PS to penetrate the cell walls of Gram (-) bacteria to gain access to sensitive intracellular sites. Multi-antibiotic resistant strains are as easily killed as wild-type strains. We have generated preliminary data using luminescent bacteria and a low-light imaging camera, that PDT will kill both Gram (-) species (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram (+) species *Staphylococcus aureus* in vivo in animal models of both early and established infections. In the case of the invasive *P. aeruginosa* mice are cured of an otherwise fatal infection. Localized PDT may have an additional advantage in that it is also possible to inactivate secreted extracellular virulence factors that pathogenic bacteria use to establish infections and invade tissue. This project will seek to explore the determinants of PDT for localized infections. Four specific aims will focus on optimizing the treatment in different mouse models of early, acute and chronic infections, comprising excisional wounds, established soft tissue infection, chronic abscesses, burns and urinary tract infections. Since one of the advantages of PDT is its rapidity compared to traditional antibiotic therapy, we will also study the use of PDT to quickly reduce the bacterial burden in the infection, followed by antibiotics to eliminate the residual bacteria.

Grant: 1R01AI051332-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: HAGMAN, KAYLA E PHD
Title: Decorin-binding proteins of *Borrelia burgdorferi*
Institution: UNIVERSITY OF TEXAS SW MED DALLAS, TX
CTR/DALLAS
Project Period: 2002/12/15-2007/11/30

DESCRIPTION (provided by applicant): Lyme disease continues to represent a major public health problem, and many aspects of Lyme disease pathogenesis and immunology thus warrant further investigation. Decorin-binding protein A (DbpA), a membrane lipoprotein of *Borrelia burgdorferi*, has been implicated in subserving the parasitic strategy of *B. burgdorferi* by functioning as a cell matrix-binding adhesin during mammalian tissue invasion. DbpA also has emerged as the most prominent new human Lyme disease vaccine candidate. However, many features of DbpA, such as its temporal expression, membrane topology, role in *B. burgdorferi* virulence, and overall utility as a protective immunogen, remain poorly understood. The current study addresses these important information gaps. To this end, the Specific Aims of this proposal are: (1) To examine the temporal expression pattern(s) of DbpA by tick-transmitted *B. burgdorferi* in the mammalian (mouse) host, (2) To examine the membrane topology of DbpA expressed by tick-transmitted *B. burgdorferi* in the mammalian (mouse) host, (3) To construct a DbpA-deficient mutant of virulent *B. burgdorferi* by insertional inactivation of *dbpA*, with emphasis on examining the role of DbpA expression in *B. burgdorferi* infectivity, virulence, and disease pathogenesis, and (4) To examine further the overall efficacy of DbpA as a vaccinogen in the mouse model of Lyme borreliosis. Regarding the latter, emphasis will be placed on (a) purifying recombinant DbpA (as immunogen) to preserve its native conformation and (b) tick infestation (challenge route for *B. burgdorferi*) of DbpA-immunized mice to mimic natural *B. burgdorferi* transmission. The current proposal represents a comprehensive study of the role of DbpA in both the pathogenesis of Lyme disease and its potential as a human Lyme disease vaccine.

Grant: 1R01AI051333-01A1
Program Director: HALL, ROBERT H.
Principal Investigator: KLOSE, KARL E
Title: Environmental Modulation of ToxT-dependent Transcription
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR SAN SAN ANTONIO, TX
ANT
Project Period: 2002/12/01-2007/11/30

DESCRIPTION (provided by applicant): Cholera is an often-fatal diarrheal disease caused by the bacterium *Vibrio cholerae*. This disease remains a health threat for the majority of the world, causing thousands of deaths every year. We have recently demonstrated that ToxT, the primary transcriptional activator of virulence genes in *V. cholerae*, is negatively regulated by certain environmental signals, and specifically by the presence of bile. Our studies will focus on dissecting the molecular mechanism(s) of environmental modulation of ToxT transcriptional activity, utilizing bile as an environmental modulatory factor. We wish to understand and exploit this negative regulation to develop novel means to prevent cholera. Essentially nothing is known about the structure/function of ToxT, so these studies also include the elucidation of the functions of the ToxT protein. Our approach first involves characterizing the domain structure of ToxT. This will be accomplished by (i). construction and characterization of chimeric ToxT proteins, and (ii). identification of ToxT amino acids important for DNA binding and transcriptional activation. Further characterization of ToxT will include the identification of all the ToxT-regulated genes of *V. cholerae* by microarray analysis, and the characterization of the ToxT DNA binding site(s). Once we have a more thorough understanding of ToxT, we will determine the mechanism of modulation of ToxT transcriptional activity by environmental signals, utilizing bile as the modulatory factor. These studies include: (i). determination of the effect of the porins OmpU and OmpT (which are known to be differentially permeable to bile) on bile modulation of ToxT activity, (ii). identification of additional *V. cholerae* genes involved in bile regulation of ToxT activity, (iii). Identification of ToxT amino acids necessary for bile regulation, and (iv). determination of the effects of bile on ToxT DNA binding activity. Finally, the relevance of environmental modulation of ToxT activity (by bile or other stimuli) will be assessed by testing the virulent properties of *V. cholerae* strains containing mutations that affect various aspects of ToxT transcription. Our ultimate goal is to learn how to manipulate ToxT by external factors in order to repress virulence gene expression and prevent cholera, i.e., to force *V. cholerae* to prevent itself from causing disease. This fundamentally different approach to cholera therapy could lead to novel antimicrobial strategies mimicking the effects of bile.

Grant: 1R01AI051356-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: CLAYBERGER, CAROL A
Title: M Tuberculosis CTL Epitopes: Vaccine Design/Evaluation
Institution: STANFORD UNIVERSITY STANFORD, CA
Project Period: 2003/02/15-2008/01/31

DESCRIPTION (provided by applicant): Tuberculosis (TB) is the leading cause of death from a single infectious agent (*Mycobacterium tuberculosis* (Mtb)), causing approximately 3,000,000 deaths each year. Although TB can be effectively treated with a combination of antibiotics, drug resistant Mtb strains have recently emerged which are classified as Category C biological agents. Thus, it is widely felt that the long term control of TB will require the development of a more effective vaccine. *Mycobacterium bovis* Bacille Calmette-Guerin (BCG), the current anti-TB vaccine, is quite variable in its ability to protect against TB but is effective against tuberculosis meningitis, suggesting that for the foreseeable future, new TB vaccines will be given as an adjuvant or boost to BCG. Thus, understanding the immune response to both Mtb and BCG is critical for the development of an improved vaccine for TB. An increasing body of evidence indicates that both CD4+ and CD8+ T lymphocytes are critical to a protective immune response against Mtb. However, little is known about the antigens targeted by protective immune responses against Mtb in humans. Such information is required for the rational development and clinical evaluation of new, more effective TB vaccines. We propose here to characterize the human CD4+ and CD8+ T cell response to a panel of Mtb antigens in order to identify correlates with protective immunity. Antigens to be tested include proteins as well as peptide epitopes restricted by HLA-A2, an allele expressed by approximately 50% of the population. Some of these proteins and epitopes were selected from a subset of Mtb genes that are highly expressed under specified conditions and whose products are predicted to localize to the extracellular milieu, while the remainder represent previously identified HLA-A2 restricted epitopes. The T cell response to these antigens will be evaluated in peripheral blood leukocytes from three different groups of BCG immune and/or Mtb infected individuals: i. Neonates immunized a birth with one of 4 strains of BCG; ii. Individuals infected with Mtb but who do not progress to disease (latent TB infected individuals); and iii. PPD+ TB patients and PPD- "anergic" TB patients. Some of these peptide epitopes will be used to develop epitope oligomers which will be used to analyze anti-Mtb responses *In vitro* and *in vivo*. Lastly, the localization and function of Mtb peptide specific memory T cells will be studied *in vivo*. Correlates of protective immunity can be used to identify or prioritize protective antigens and vaccine candidates, to optimize vaccine dosing, schedules, adjuvants, etc., and to provide early evidence of efficacy. For TB, which takes years to decades to develop after infection with Mtb, immune correlates with protection are an attractive, and perhaps essential, supplement to efficacy trials.

Grant: 1R01AI051360-01A1
Program Director: PERDUE, SAMUEL S.
Principal Investigator: O'TOOLE, GEORGE A BS
Title: Mechanisms of Biofilm Antibiotic Resistance
Institution: DARTMOUTH COLLEGE HANOVER, NH
Project Period: 2003/04/01-2008/03/31

DESCRIPTION (provided by applicant): Biofilms are complex bacterial communities attached to a surface. The formation of biofilms has a profound impact on human health and industrial processes and has been recognized as an important, but poorly studied aspect of the bacterial life cycle. Biofilm development includes a transition from individual, planktonic (free-swimming) bacteria to a mode of life attached to a surface as part of a multi-cellular community, and the subsequent return to a planktonic existence. One of the most widely recognized properties of bacteria growing in a biofilm is their increased resistance to antimicrobial agents. Despite increasing interest in understanding how resistance develops, little is known about the molecular mechanisms that drive biofilms cells towards this highly resistant state. Our evidence supports the central hypothesis of this proposal: antimicrobial resistance of biofilm-grown cells requires distinct genetic elements. Herein, we propose a series of complimentary approaches to elucidate molecular mechanisms underlying biofilm-specific antibiotic resistance using three model antibiotics. While part of the proposed work will continue our efforts to study known biofilm-specific functions in *P. aeruginosa* (Specific Aim I), work described in Specific Aims II and III will begin in depth analysis of two newly discovered genetic loci required for resistance to antibiotics in a biofilm. The multiple experimental approaches outlined in this proposal should lead to a better understanding of the mechanism(s) involved in development of biofilm-specific antibiotic resistance, and potentially to new therapeutic strategies for modulating these properties. Specific Aim I. Studies of known genes in biofilm-specific antibiotic resistance. Specific Aim II. Determine the role of a glucan synthetase enzyme in the development of biofilm-specific antibiotic resistance. Specific Aim III. Studies of the role of a putative new efflux pump in biofilm-specific antibiotic resistance.

Grant: 1R01AI051407-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: COBURN, JENIFER L PHD
Title: Borrelia-Integrin Interactions
Institution: NEW ENGLAND MEDICAL CENTER BOSTON, MA
HOSPITALS
Project Period: 2003/01/01-2007/12/31

DESCRIPTION (provided by applicant): *Borrelia burgdorferi*, the agent of Lyme disease, is an obligate parasite of its mammalian reservoirs and of the tick vectors that transmit the bacteria. *B. burgdorferi* infection can affect the skin, joints, heart, and nervous system, and can withstand the onslaught of the innate and acquired immune systems. Interactions with mammalian cells are likely to be important in this complex infection process, and *B. burgdorferi* binds to three members of the integrin family of mammalian cell-surface receptors. The *B. burgdorferi* protein p66 is a ligand for integrins α IIb β 3 and α v β 3, but does not bind integrin α 5 β 1, suggesting that other ligands are expressed by the spirochete. P66 is expressed by *B. burgdorferi* in the mammal, but not in the tick, supporting its potential importance in mammalian infection. To further study the expression of p66, the importance of p66 in the biology of mammalian infection by *Borrelia*, and to identify additional *B. burgdorferi* proteins that bind to integrins, three Specific Aims will be pursued: Aim 1. In vitro and in vivo analysis of *B. burgdorferi* mutants that do not express p66. P66-deficient *B. burgdorferi* will be compared to wild type with regard to growth and ability to establish infection. Aim 2. Analysis of the regulation of *B. burgdorferi* p66 expression. The pattern of p66 expression suggests that it is a paradigm for *B. burgdorferi* genes that are important in the mammalian host, so genetic and biochemical approaches will be taken to identify *B. burgdorferi* modulators of p66 expression. Aim 3. Characterization of potential integrin ligands identified by analysis of the *B. burgdorferi* genome sequence. Five *B. burgdorferi* proteins that contain integrin-recognition motifs and secretion signals will be tested for integrin-binding, localization on the bacterial surface, and for roles in infection. These experiments will shed light on the importance of p66 and integrin attachment in the ability of *B. burgdorferi* to cause infection, and on regulation of gene expression in this important pathogen. The long-term goal of this work is to understand the pathogenesis of *B. burgdorferi* infection, which is an important model for how certain bacteria co-opt the biology of their immunocompetent hosts to cause disease.

Grant: 1R01AI051472-01A1
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: GILL, STEVEN R PHD
Title: Comparative Genomics of the Chlamydiaceae
Institution: INSTITUTE FOR GENOMIC RESEARCH ROCKVILLE, MD
Project Period: 2003/07/01-2007/12/31

DESCRIPTION (provided by applicant): We intend to extend genomics from the characterization of individual bacterial genomes to characterizing the evolution of infectious disease-causing bacteria. The Chlamydiales, an order of Gram-negative obligate intracellular pathogens, are ideally suited to this approach. Members of this order cause a range of diseases but continue to be refractory to conventional genetic analyses. Our novel, multifaceted approach, which we have named "Taxogenomics," is to first sequence the type strains of the Chlamydiaceae species not addressed by previous or current projects - *C. suis*, *C. pecorum*, and *C. psittaci* (a potential biological warfare agent). Additionally, we will sequence the koala strain of *C. pneumoniae* and the most evolutionarily distant members of the Chlamydiales, *Waddlia chondrophila* and *Simkania negevensis*. To identify those genes which may have been lost by the laboratory adapted type strains or been acquired by "wild" isolates, we will apply subtractive hybridization by pooling multiple isolates from each species and subtracting those genes shared by the type strain. With sequences from representatives of all genomes and with sequences derived from the taxonomic genomic subtractions, we will then create a gene database of all known ORFs from this evolutionarily isolated family. We will use this database to design a non-redundant microarray of Chlamydiales gene variation (called here the "Taxochip"). In this study, we will use the Taxochip in a suite of comparative genomic hybridization (CGH) experiments using a unique set of diverse Chlamydiales isolates that we have compiled from around the globe. We expect to use this genome data to investigate the evolution of the Chlamydiales and identify those genes that may differentiate the variety of observed host/tissue niches and resulting disease outcomes. We also will assess the potentially important role of horizontal gene transfer in creating novel pathogenicity phenotypes and the likely important relationship between animal and human chlamydial strains. Additionally, the Taxochip design and the Chlamydiales gene database will be of long-term benefit to all chlamydiologists and molecular evolutionists who are increasingly using the Chlamydiales as a standard model for studying reductive evolutionary processes. This study is designed to address broad questions that deal with the genetic and evolutionary basis of the development of intracellular parasitism and pathogenicity in the Chlamydiales. With broad and international support from the scientific community, we consider this a groundbreaking proposal and anticipate it will be a benchmark study in comparative microbial genomics.

Grant: 1R01AI051477-01A1
Program Director: KLEIN, DAVID L
Principal Investigator: LAFONTAINE, ERIC R PHD
Title: Adherence mechanisms of *Moraxella catarrhalis*
Institution: MEDICAL COLLEGE OF OHIO AT TOLEDO TOLEDO, OH
Project Period: 2002/12/01-2007/11/30

DESCRIPTION (provided by applicant): Long considered a harmless commensal of the human respiratory tract, the gram-negative bacterium *Moraxella catarrhalis* has recently emerged as a significant cause of infectious diseases. These infections are a significant health problem, medically as well as economically, and addressing these issues is complicated by the fact that little is known about pathogenesis by *M. catarrhalis*. Our long-term objectives are to study the molecular basis for *M. catarrhalis* adherence to its human host, and to evaluate the potential of interfering with this adherence as a strategy to reduce the risks of infections. We have already isolated *E. coli* recombinant clones that gained the ability to bind to human cells by virtue of expressing new *M. catarrhalis* adhesin genes. Furthermore, we have also isolated fourteen *M. catarrhalis* transposon mutants that are substantially reduced in their ability to bind to human lung cells. The Specific Aims of the Proposed Research Plan are: 1. To identify and characterize the genes encoding new *M. catarrhalis* adhesins for human cells. 2. To evaluate the vaccinogenic potential of these new *M. catarrhalis* adhesins. 3. To identify *M. catarrhalis* gene products involved in the expression as well as surface display of adhesins. We will use recombinant DNA techniques as well as transposon mutagenesis to identify genes that are involved in the binding of *M. catarrhalis* to human cells. We will determine the nucleotide sequence of candidate genes and characterize their encoded products. We will also use recombinant forms of new *M. catarrhalis* adhesins to determine whether they elicit the production of biologically relevant antibodies. Our studies will have direct applications in vaccine as well as antimicrobial development.

Grant: 1R01AI051486-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: SAMUELS, D SCOTT PHD
Title: Regulation of gene expression in *Borrelia burgdorferi*
Institution: UNIVERSITY OF MONTANA MISSOULA, MT
Project Period: 2003/09/05-2007/12/31

DESCRIPTION (provided by applicant): The bacterium *Borrelia burgdorferi* is a causative agent of Lyme disease. *B. burgdorferi* can synthesize several different outer surface proteins that are involved in pathogenesis or transmission during the enzootic cycle. The regulation of outer surface protein synthesis is not well understood at the molecular level. This application proposes to evaluate the hypothesis that cis-acting factors, such as DNA supercoiling, and trans-acting factors, such as DNA-binding repressor proteins, regulate the expression of outer surface protein genes in response to environmental signals. Production of outer surface proteins OspA and OspC is reciprocally regulated, which may be how *B. burgdorferi* adapts to the different environments of the tick vector and mammalian host or effects transmission between the environments. An ospAB operon promoter-specific trans-acting protein is hypothesized to repress ospAB transcription. The regulatory protein will be purified and identified. Cis-acting sequences in the ospAB promoter region are hypothesized to mediate transcriptional regulation. These sites will be mapped and characterized. The architectural DNA-binding protein Hbb is hypothesized to facilitate regulation of ospC expression. The function of Hbb will be probed by mutagenesis of the hbb gene and the Hbb binding site in the ospC promoter region. The ospC promoter will be replaced with an inducible promoter system to control cellular OspC levels without perturbing DNA supercoiling so that the coupling of ospC and ospAB transcription can be studied. ospC gene expression from the flac hybrid promoter is hypothesized to be regulated by IPTG and to influence ospAB operon expression. The mechanism by which DNA supercoiling regulates ospC expression is hypothesized to involve Hbb and specific sequence motifs in the ospC promoter region. Mutant ospC promoters will be constructed. The transcriptional response of these mutants to temperature and DNA supercoiling will be assayed in order to define the cis-acting elements responsible for regulation. The long-term objective of these studies is to understand the mechanism of outer surface protein gene regulation in response to environmental signals.

Grant: 1R01AI051490-01A1
Program Director: HALL, ROBERT H.
Principal Investigator: FULLNER, KARLA J
Title: Genetic and biochemical study of V. cholerae RTX toxin
Institution: NORTHWESTERN UNIVERSITY EVANSTON, IL
Project Period: 2002/12/15-2007/11/30

DESCRIPTION (provided by applicant): *Vibrio cholerae* strains that do not produce cholera toxin induce a more inflammatory diarrhea than normal cholera disease, implicating other potent toxins in the pathogenesis of cholera. Several accessory toxins of *V. cholerae* are purported to account for this reactogenic response. One of these factors is the newly discovered VcRtxA toxin of *V. cholerae*. VcRtxA is a large protein toxin that is a unique member of the RTX family. Production of this toxin has been evolutionarily conserved by *Vibrio* sp indicating that maintenance of this large toxin is essential for virulence or survival in the environment. Its cytotoxicity has been further shown to function by a novel mechanism. This toxin causes depolymerization of actin stress fibers in both polarized and non-polarized cell lines by a unique pathway. Concurrent with depolymerization, the actin molecules become covalently linked together into dimers, trimers, and higher order multimers. This observation suggests that covalent crosslinking of actin by the toxin drives actin depolymerization. This unusual reaction distinguishes VcRtxA from all other bacterial toxins that cause actin depolymerization. Research performed under this grant proposal will investigate further the novel biochemical properties of this important virulence factor. The VcRtxA toxin is the largest single polypeptide protein toxin ever described, however, it is unclear whether the full 4545 amino acid protein is the toxic moiety. The size of the toxic moiety and potential post-translational modifications will be identified by examining biochemical properties of the active form of purified toxin. The catalytic activity will then be investigated and the role of a putative catalytic domain in this reaction will be assessed. It will then be established whether the toxin is active at the membrane or within the cytoplasm of the target cells, and the role of a putative actin-binding domain will be characterized.

Grant: 1R01AI051518-01A1
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: KAWULA, THOMAS H PHD
Title: Mechanisms of Immune Avoidance by Haemophilus ducreyi
Institution: UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC
HILL
Project Period: 2003/04/01-2008/03/31

DESCRIPTION (provided by applicant): Chancroid is a bacterial genital ulcer disease endemic to sub-Saharan Africa and Eastern Asia. The immune response to Haemophilus ducreyi, the etiologic agent of chancroid, is not very effective at either clearing organisms from an infected individual or at preventing subsequent H. ducreyi re-infections. The three specific aims described herein are designed to utilize and extend the swine model of chancroid to test hypotheses that relate to the mechanisms underlying this poor immune response. Aim 1 assesses the host response to H. ducreyi by testing the hypothesis that H. ducreyi elicit predominantly cellular immunity, which is ineffective at clearing the infecting bacteria. The cytokine expression pattern of H. ducreyi specific CD4 and CD8 lymphocytes isolated from infected lesions will be examined. In Aim 2 the bacterial contribution to inhibiting the immune response will be addressed by testing the hypothesis that the H. ducreyi CDT and hemolysin toxins block immunity development through their cytotoxic effect on immune effector cells. This aim will be accomplished by comparing the kinetics and strength of immune development following inoculation with cdt/hem double mutants and wild type H. ducreyi, while also assessing differences in T cell cytokine expression and antigen specificity effected by mutant as compared to wild type organisms. The specific contribution of the toxins on the immune response will be examined by testing their effect on the development of antibody and cytotoxic T cells to hen egg lysozyme. Aim 2 addresses the hypothesis that a vigorous bactericidal antibody response to H. ducreyi would result in more efficient clearing of organisms from infected tissue and prevent subsequent H. ducreyi re infection. An H. ducreyi outer membrane protein that is a target for bactericidal antibody has been identified. This protein will be tested for the ability to elicit a protective immune response to H. ducreyi.

Grant: 1R01AI051520-01A1
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: NILLES, MATTHEW L
Title: Protein Interactions in Type III Secretion in *Y. pestis*
Institution: UNIVERSITY OF NORTH DAKOTA GRAND FOLKS, ND
Project Period: 2003/02/01-2008/01/31

DESCRIPTION (Provided by applicant): *Yersinia pestis*, the causative agent of Plague, harbors a -71 KB plasmid that encodes many virulence-related proteins. Among the pCDI-encoded genes are genes specifying for the construction of a type III secretion system and the genes for the secreted effector proteins (collectively termed Yops). Type III secretion systems allow pathogenic bacteria to specifically translocate effectors into eukaryotic cells for the pathogen's benefit. The Yops exert their effects on targeted eukaryotic cells that results in a blockage of phagocytosis by immune cells. This effect allows *Y. pestis* to remain extracellular during the course of an infection. In *Y. pestis* the activity of the type III system is controlled by environmental stimuli, in vitro secretion is stimulated by the removal of calcium ions. In vivo Yops translocation is triggered by contact with eukaryotic cells. Appropriate control of type III secretion is required for the disease process as mutants defective in control are avirulent. Control of type III secretion relies on both positive and negative control circuits. LcrG and LcrV are two proteins whose interaction is required to control type III secretion in *Y. pestis*. LcrV is required to activate secretion while LcrG is required to inactivate secretion. Their interaction provides a linkage between positive and negative regulation in type III secretion control. The mechanism responsible for their controlling effects are largely unknown, although progress has been made. Published studies have shown that the interaction of LcrG and LcrV is required to control secretion. Additionally, a separate study demonstrated that a ratio of LcrV to LcrG, favoring LcrV, is required to activate secretion. Clearly, more work is needed to define the roles of LcrG and LcrV in the activation of secretion. Specifically, in this proposal we shall: Aim 1. Define structure-function relationships within LcrG relating to LcrV interaction and secretion-blocking. Aim 2. Screen for other proteins that may interact with LcrG. Aim 3. Refine the subcellular localization of LcrG. Aim 4. Examine mechanisms that alter LcrG levels relative to LcrV. Type III secretion systems have emerged recently as a common theme in the pathogenic mechanism of many gram-negative bacterial pathogens. Work in the yersiniae has been at the forefront of research on the characterization and function of type III mechanisms. An understanding of how these systems are controlled should lead to a deeper understanding of the intimate and dynamic interactions between pathogens and their hosts.

Grant: 1R01AI051533-01A1
Program Director: AULTMAN, KATHRYN S.
Principal Investigator: DOBSON, STEPHEN L PHD
Title: Vector Population Modification Using Wolbachia Symbionts
Institution: UNIVERSITY OF KENTUCKY LEXINGTON, KY
Project Period: 2002/12/01-2006/11/30

DESCRIPTION (provided by the applicant): Intracellular Wolbachia bacteria infect a diverse range of invertebrate hosts, including medically important disease vectors. Many Wolbachia infections cause cytoplasmic incompatibility, which effectively sterilizes mating between host individuals that harbor differing infection types. We have recently developed a model that defines the relationship between the Wolbachia infection frequency and host population size. Model simulations predict a novel strategy for suppressing insect populations. This strategy is based upon artificially prolonging an unstable coexistence that occurs when two or more incompatible Wolbachia types infect a host population, resulting in host population suppression. As Wolbachia occurs naturally within a broad range of invertebrates, this strategy is potentially applicable to a variety of medically and economically important systems. In addition to population suppression strategies, population replacement strategies propose to employ Wolbachia as a vehicle for spreading desired genotypes (e.g., refractoriness to disease transmission) into vector populations. In the outlined research, we will develop, test and evaluate population suppression and replacement strategies predicted by model simulations in populations of the vector mosquito *Culex pipiens*. *Cx. pipiens* has been selected as the initial target due to its importance as a disease vector and the availability of multiple, incompatible Wolbachia infections in naturally occurring *Culex* populations. Additional experiments will examine strategies for retarding, arresting or reversing Wolbachia-based population replacement. The latter strategies represent an appropriate safeguard that may prove necessary if unexpected, negative consequences become associated with population replacement. To improve strategy design, implementation, and evaluation, aims will include better defining the infection dynamics of the *Culex*/Wolbachia symbiosis (e.g., maternal transmission rates and Wolbachia effects on host fitness), simplifying discrimination between *Culex* infection types, and improving methods for field cage evaluation of Wolbachia control strategies.

Grant: 1R01AI051561-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: KERNODLE, DOUGLAS S
Title: Pro-Apoptotic Tuberculosis Vaccine
Institution: VANDERBILT UNIVERSITY NASHVILLE, TN
Project Period: 2003/04/01-2007/03/31

DESCRIPTION (provided by applicant): A major hurdle in the development of effective vaccines against pathogens that reside within macrophages, including *Mycobacterium tuberculosis*, is how to deliver antigens in a manner that stimulates a protective cellular immune response. Recent investigations involving antisense mutants of *M. tuberculosis* that have diminished production of iron-cofactored superoxide dismutase (SOD) show that they are attenuated, induce strong CD4+ and CD8+ T-cell responses in mice, and exhibit promising activity as a vaccine prototype. These effects appear to be related to an unmasking of the innate immune responses normally inhibited by SOD, which is a prominent extracellular enzyme of *M. tuberculosis* and other pathogenic mycobacteria. The enhanced innate host immune responses presumably permit apoptosis-associated cross-presentation of microbial antigens via MHC Class I pathways to induce strong adaptive CD4+ and CD8+ T-cell responses, in contrast to the current vaccine for tuberculosis, BCG, which exhibits a predominant CD4+ T-cell response and minimal CD8+ T-cell responses. The goals of the current proposal are first, to characterize the cellular and cytokine responses in the lung observed early after infection with SOD-diminished *M. tuberculosis*, as rapid pulmonary interstitial infiltration with mononuclear cells undergoing apoptosis appears to be a process unique to the SOD-diminished strains that is not observed during infection with either virulent *M. tuberculosis* or BCG. This should define the conditions under which antigen cross-presentation occurs in vivo, yielding information that may be useful for a variety of vaccines. The second goal is to construct non-reverting SOD-diminished mutants of H37Rv and BCG by replacing the wild-type SOD allele with mutant alleles, some of which encode enzymatically less efficient mutants of SOD. This should yield a SOD-diminished vaccine candidate that is stable and safe enough for administration to man. The third goal is to determine the optimal level of SOD production for maximal vaccine efficacy and the immune correlates of protection. Diminishing the production of factors produced by intracellular pathogens that inhibit macrophage apoptosis is a strategy for making new vaccines that achieve MHC Class I antigen presentation. This should have implications not only for tuberculosis but also for other infectious diseases in which CD8+ T-cell responses are a critical component of a protective immune response.

Grant: 1R01AI051588-01A1
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: SCHWEIZER, HERBERT P PHD
Title: Regulation and function of Pseudomonas drug efflux pumps
Institution: COLORADO STATE UNIVERSITY-FORT FORT COLLINS, CO
COLLINS
Project Period: 2003/05/01-2007/04/30

DESCRIPTION (provided by applicant): *Pseudomonas aeruginosa* is an opportunistic pathogen that causes a multitude of infections, which are difficult to treat because of this bacterium's intrinsic and acquired antibiotic resistance. This antibiotic resistance can be attributed to synergy between a low-permeability outer membrane (OM) and active efflux from the cell. The genome of *P. aeruginosa* encodes 35 proposed drug efflux systems belonging to five different families, but our working hypothesis is that the clinically relevant efflux pumps contributing to intrinsic and/or acquired drug resistance belong to the resistance nodulation division (RND) family which contains 12 members. Genome sequence and expression analysis of these systems poses several questions that remain unanswered. First, because only a subset of the RND operons contains genes for OM channel proteins, the question is whether these systems must recruit other OM channels from elsewhere on the genome to function as tripartite efflux systems for drug efflux across the entire cell envelope, as current dogma says, or whether they can function as two-component systems for other substrates. Second, with the exception of a single pump, the other RND pumps are tightly regulated but the regulatory networks governing their expression and the inducing substrates remain unknown. Our other working hypotheses therefore are: (i) efflux pumps not encoding their own OM channels may either recruit hitherto unidentified proteins for function with certain substrates but may also function as two-component efflux systems for other substrates; (ii) Efflux pump expression is governed by specific and probably also global transcriptional regulators. We will test these hypotheses using the recently discovered MexJK efflux pump that is normally silent and does not encode its own OM channel. We also propose to amend these studies to probe whether any global transcriptional regulators may be involved in RND efflux pump expression. Specifically we propose to: Aim 1-Establish the regulation of MexJK by its cognate regulator MexL using biochemical and molecular studies; identify an inducer that may be present in certain growth media; identify a putative activating factor present in *P. aeruginosa* but absent in *E. coli*. Aim 2: Establish the molecular architecture of MexJK, specifically its OM membrane channel requirement, if any, using genetic and biochemical methods. Aim 3: Probe other, perhaps global regulators of efflux operon expression, specifically MexS and PA4878.

Grant: 1R01AI051675-01A1
Program Director: RUBIN, FRAN A.
Principal Investigator: FOXMAN, BETSY
Title: GBS: Epidemiologic Characterization for Gene Discovery
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2003/01/01-2007/12/31

DESCRIPTION (provided by applicant): Group B Streptococcus (GBS) is a major cause of neonatal sepsis and meningitis. First emerging as a pathogen in newborn nurseries in the 1970s, it is now a pathogen of concern in nursing homes, intensive care units and outpatient settings. Little is known about GBS virulence factors. The overall goals of this application are 1) to use genetic fingerprinting techniques to better describe the molecular epidemiology of GBS of several epidemiologically defined collections; and 2) to identify new genes associated with GBS disease. By combining epidemiologic information with molecular genetics, we can maximize our ability to detect GBS virulence-related genes associated with invasive disease, extra-intestinal colonization, transmission and antibiotic resistance. This strategy also gives us insight as to the relative importance of the identified genes and their potential mechanism. A better understanding of the molecular epidemiology and the identification of new GBS virulence genes will facilitate the discovery of new therapies and prevention strategies for GBS disease.

Grant: 1R01AI051735-01A1
Program Director: SCHMITT, CLARE K.
Principal Investigator: WELCH, RODNEY A
Title: StcE, an E.coli O157:H7 Protease Specific for C1-Inh
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 2003/01/16-2007/12/31

DESCRIPTION (Provided by applicant): Enterohemorrhagic Escherichia coli (EHEC), principally serotype O157:H7, cause an estimated 20,000 cases of diarrheal disease in the United States per year. 2-6 percent of the infected individuals, mostly young children progress to a severe renal disease, hemolytic uremic syndrome (HUS). The EHEC pathogenic factors that lead to bloody colitis and HUS are poorly understood, but knowledge of some mechanisms has recently emerged. Intimin-mediated adherence and type III effectors are encoded by a chromosomal locus termed LEE. The phage-encoded Shiga toxins (Stxs) are responsible for significant aspects of EHEC disease. EHEC strains commonly possess large plasmids, the prototype being pO157. We have identified a new pO157 gene, stcE, which encodes an extracellular zinc-metalloendoprotease (ZMP) that specifically cleaves the critical anti-inflammatory regulator C1-esterase inhibitor (C1-Inh). C1-Inh is a serine protease inhibitor (serpin) that provides the principal inhibition of the proteolytic cascades involved in classic and mannan-binding ligand complement activation, contact activation and intrinsic coagulation. C1-Inh inhibits diverse proteases: C1r and C1s, MASP-1, MASP-2, kallikrein, FXIIa, FXIa, and plasmin. Deficiencies in C1-Inh cause profound clinical syndromes. The best known is hereditary angioedema (HAE), a genetic deficiency in C1-Inh, which is characterized by transient, recurrent attacks of intestinal cramps, vomiting, diarrhea and life-threatening episodes of tracheal swelling. Fluorescent StcE binds to cultured macrophages, B- and T-cells. Thus, StcE is an example of a growing class of ZMPs such as tetanus, botulinum and anthrax lethal factor toxins. These ZMPs, in contrast to the homologous Pseudomonas and Vibrio ZMPs, have specific, non-extracellular matrix protein targets. We will test the hypothesis that StcE degrades soluble or cell-associated C1-Inh, and this results in poorly regulated serine protease cascades involving complement activation, contact activation and coagulation. This dysregulation would then contribute to local inflammation, tissue damage and edema. The elucidation of StcE structure and function(s) may result in new targets for chemotherapeutic or immune prevention or treatment of EHEC infections, which now are best managed only by supportive therapy.

Grant: 1R01AI052141-01A1
Program Director: RUBIN, FRAN A.
Principal Investigator: NEELY, MELODY N BS
Title: Streptococcal-Zebrafish Model of Bacterial Pathogenesis
Institution: WAYNE STATE UNIVERSITY DETROIT, MI
Project Period: 2003/02/15-2007/01/31

DESCRIPTION (provided by applicant): Streptococcal pathogens continue to evade concerted efforts to decipher clear-cut virulence mechanisms, although numerous genes have been implicated in pathogenesis. A single species can infect a diversity of tissues, suggesting the expression of specific virulence factors based on the local tissue environment or stage of infection. Our long-range goal is to identify the interactions that occur between the host and pathogen that lead to activation of virulence mechanisms and contribute to specific streptococcal disease states. The objective of this application is to characterize specific virulence mechanisms utilized within various tissues in vivo by employing a unique animal model, the zebrafish (*Danio rerio*). We will accomplish this by studying infection by two streptococcal species that represent two forms of streptococcal disease: a natural pathogen of both fish and humans, *Streptococcus iniae*, and a human-specific pathogen, *Streptococcus pyogenes*. While *S. iniae* primarily causes systemic disease in the zebrafish following intra-muscular injection, *S. pyogenes* causes a locally spreading necrotic disease confined to the muscle. By studying pathogens that are virulent for both fish and humans and that mediate disease states in the zebrafish that are identical to those found in human streptococcal infections, we will be able to identify common virulence strategies shared by a number of Gram positive pathogens. The central hypothesis is that streptococcal pathogens respond to their host by initiating specific virulence mechanisms based on the local tissue environment or host-specific factors expressed within that tissue. We propose to: (1) identify and characterize bacterial proteins that interact with the host in vivo to cause specific disease states and (2) characterize the role in pathogenesis of proteins previously implicated in virulence.

Grant: 1R01AI052148-01A1
Program Director: SCHMITT, CLARE K.
Principal Investigator: DARWIN, ANDREW J PHD
Title: The Psp response of *Yersinia enterocolitica*
Institution: NEW YORK UNIVERSITY SCHOOL OF NEW YORK, NY
MEDICINE
Project Period: 2003/03/15-2008/02/29

DESCRIPTION (provided by applicant): Bacteria of the genus *Yersinia* are responsible for a variety of human diseases. *Y. pestis* causes Bubonic Plague, and has recently regained prominence in public awareness due to its potential use as an agent of bioterrorism. In contrast, *Y. pseudotuberculosis* and *Y. enterocolitica* cause primarily gastrointestinal disease. However, despite the differences in disease symptoms, the three pathogenic *Yersinia* species are closely related, and share several common virulence determinants. *Yersinia* studies have provided fundamental insights into bacterial pathogenesis, including the first example of the widespread type III secretion system (TTSS). In *Yersinia*, as in all bacterial pathogens, many of the proteins that play important roles in virulence, including components of the TTSS, are located in the cell envelope. Under certain conditions, some envelope proteins become misfolded/mislocalized. Specific stress-response mechanisms deal with this problem, examples of which are the RpoE and Cpx systems of *Escherichia coli* and related organisms. These extracytoplasmic stress responses play important roles during host infection. The central hypothesis of this proposal is that a different extracytoplasmic stress response system is encoded by the phage-shock-protein locus (psp) of *Y. enterocolitica*. A *Y. enterocolitica* psp mutant is avirulent, and homologous psp loci are found in other bacterial pathogens, including *Y. pestis* and *Vibrio cholerae*. Our preliminary data indicate that the Psp system responds to mislocalization of several envelope proteins involved in virulence, including at least one component of a TTSS. By studying the Psp system we will gain further insight into the essential ability of bacteria to respond to stressful conditions that occur during host infection. Specifically, we propose to: (1) Analyze the proteins that induce the Psp system and characterize any overlap between Psp inducers and RpoE/Cpx inducers; (2) Determine the topology of the Psp system, and investigate how Psp allows extracytoplasmic stress to be sensed and signaled across the cytoplasmic membrane; (3) Characterize genes directly controlled by the Psp system, in order to identify further stress response components.

Grant: 1R01AI052151-01A1
Program Director: HALL, ROBERT H.
Principal Investigator: BOOR, KATHRYN J MS
Title: Sigma B and stress response in *Listeria monocytogenes*
Institution: CORNELL UNIVERSITY ITHACA ITHACA, NY
Project Period: 2003/08/01-2007/01/31

DESCRIPTION (provided by applicant): *Listeria monocytogenes* (L. m.) causes serious invasive diseases in humans and animals, with a human case mortality rate of approximately 20%. One goal of the US Dept. of Health and Human Services Healthy People 2010 Initiative is to reduce human listeriosis cases by 50%. The long-term objective of our research program is to contribute to that end through identification of factors that influence L. m. pathogenesis, which ultimately will enable development of novel and effective intervention strategies for preventing listerial infections. The work proposed in this application is designed to test the specific hypotheses that (i) the sigma/B general stress response system in gram-positive bacterial pathogens (and specifically in L. monocytogenes) provides a key transcriptional regulatory mechanism that facilitates environmental survival and virulence through induction of stress response genes; and that (ii) bacterial stress response systems contribute to pathogenesis by responding to specific environments, including those encountered in the host, through initiation of stress response and virulence gene expression (e.g., prfA). The specific aims of these studies are to: (1) Define the L. m. sigmaB regulon through proteomic and genetic approaches. (2) Determine sigmaB regulon expression patterns under environmental stress conditions, sigma/B -dependent gene expression patterns will be evaluated using microarrays and reporter (-3) Measure sigmaB-dependent gene expression during host cell infection. Reporter fusions to selected sigmaB-dependent genes (e.g., prfA) in wildtype L. m. and selected null mutant strains (e.g., Δ sigB) will be used to identify gene expression patterns during cellular infection in tissue culture models. (4) Characterize Δ sigmaB mutant virulence in tissue culture and animal models. At the conclusion of these studies, we will have developed an understanding of the contribution of cyB and the sigmaB-dependent stress response system to L. monocytogenes environmental survival and infection. More broadly, L. monocytogenes will serve as a model system for examining the role of alternative sigma factor-directed general stress response systems in survival and pathogenesis of gram-positive food-borne pathogens.

Grant: 1R01AI052154-01A1
Program Director: HALL, ROBERT H.
Principal Investigator: MARQUIS, HELENE DVM
Title: Listeria phospholipase C: sorting, activation, release
Institution: CORNELL UNIVERSITY ITHACA ITHACA, NY
Project Period: 2003/09/01-2008/02/29

Listeria monocytogenes is a facultative intracellular Gram-positive bacterium that causes invasive, often fatal, disease in susceptible hosts. As a foodborne pathogen, the bacterium has become a significant public health problem and has caused several epidemics in the United States and Europe. The virulence of *L. monocytogenes* is directly related to its ability to grow in the cytosol of host cells and its efficacy in spreading from cell to cell without leaving the intracellular milieu. We are interested in the mechanism regulating the activity of a bacterial phospholipase C (PC-PLC), whose function increases the efficacy of bacterial cell-to-cell spread. PC-PLC is made as a proenzyme whose activation requires cleavage of a Nterminal prodomain. During intracellular growth, bacteria cumulate a pool of PC-PLC that is rapidly secreted in its active form upon a decrease in intracellular pH. A bacterial metalloprotease (Mpl) is involved in the regulation of PC-PLC activation and secretion. However, factors other than pH and Mpl control PC-PLC activation and secretion since a decrease in pH has little effect on the status of bacteria-associated PC-PLC in vitro. Our results reveal the existence of a previously undescribed mechanism regulating the activity of a virulence factor in a Gram-positive bacterial pathogen. This mechanism can be divided into three major steps: sorting, activation, and release of the virulence factor. Premature activation and secretion of PC-PLC in the cytosol of infected cells is cytotoxic, emphasizing the importance of this control mechanism for the intracellular survival of *L. monocytogenes*. Our ultimate goal is to define at the cellular, molecular, and biochemical levels the mechanism regulating the activity of *L. monocytogenes* PC-PLC during infection. The specific aims of this proposal are to (I) Define functional regions of *L. monocytogenes* PC-PLC and Mpl that promote their association with the bacterial cell, (II) Identify and analyze bacterial factors accessory to the regulation of PC-PLC activity, and (III) Define environmental signals influencing the regulation of PC-PLC activity. On a broader scope, these studies will contribute to our understanding of protein secretion in Grampositive bacteria.

Grant: 1R01AI052267-01A1
Program Director: TAYLOR, KATHERINE A.
Principal Investigator: HORWITZ, BRUCE H BA
Title: Inhibition of Microflora-Induced Colitis by NF-kB
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 2003/07/01-2007/12/31

DESCRIPTION (provided by applicant): The purpose of this proposal is to define the cellular and molecular mechanisms by which the NF-kappaB subunits p50 and p65 inhibit inflammation within the lower bowel. The inhibitory functions of p50 may be especially relevant to the control of inflammation within the colon, as our laboratory has shown that mice lacking p50 (p50^{-/-}) are sensitive to colitis induced by *Helicobacter hepaticus*, and this sensitivity is significantly exacerbated in mice that both lack p50 and are heterozygous for p65 (p50^{-/-}p65^{+/-}). These mice are sensitized to the development of colitis by a defect intrinsic to the innate immune system. This defect may reflect an inability to control *H. hepaticus*-induced inflammatory gene expression within antigen presenting cells (APCs), as *H. hepaticus* infection induces higher levels of the critical inflammatory cytokines IL-12p40 and IP-10 in p50^{-/-} and p50^{-/-}p65^{+/-} macrophages than in WT macrophages. The goals of this proposal are: 1) To determine the mechanism by which p50 and p65 within cells of the innate immune system contribute to inhibiting the inflammatory response to *H. hepaticus*. Using a novel mouse strain created in our laboratory (p50^{-/-}p65^{+/-}RAG-2^{-/-}), we will evaluate the possibility that p50/p65 activity is required within the innate immune system to facilitate the inhibitory function of regulatory T cells. 2) To determine the mechanisms by which p50 and p65 inhibit *H. hepaticus* induced inflammatory gene expression. We will use molecular techniques to compare the function of endogenous IL-12p40 and IP-10 promoters in WT, p50^{-/-}, and p50^{-/-}p65^{+/-} macrophages. 3) To determine whether p50 and p65 prevent an overaggressive immune response that injures the host, or alternatively, whether p50 and p65 prevent an immune deficiency that leads to increased bacterial burden. We will compare bacterial burden in RAG-2^{-/-} and p50^{-/-}p65^{+/-}RAG-2^{-/-} mice, and determine whether introduction of functional innate immune cells into p50^{-/-}p65^{+/-}RAG-2^{-/-} mice can prevent *H. hepaticus*-induced inflammation. Taken together, we believe that these studies will lead to insights regarding the molecular pathogenesis of inflammatory bowel disease that could lead to novel therapeutic strategies.

Grant: 1R01AI052291-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: NASH, KEVIN A PHD
Title: Mechanisms of macrolide resistance in mycobacteria
Institution: CHILDREN'S HOSPITAL LOS ANGELES LOS ANGELES, CA
Project Period: 2003/07/01-2005/12/31

DESCRIPTION (provided by applicant): The cornerstones for controlling infections caused by non-tuberculosis mycobacteria (NTM) are the macrolides, clarithromycin and azithromycin. The genetic basis of clinically acquired resistance to these agents is conferred by mutation in the 23S ribosomal RNA (rRNA) gene. However, we understand little of the mechanisms of intrinsic macrolide resistance of mycobacteria, particularly *M. tuberculosis*. Recent studies suggest that mycobacteria have erm methylase genes and macrolide efflux pumps, which may affect susceptibility to macrolides. Therefore, we hypothesize that there are several mechanisms that affect the antimycobacterial activity of macrolides. These mechanisms include drug efflux, expression of erm genes, and, the acquisition of a 23S rRNA gene mutation. Consequently, the long-term objective of this work is to characterize the mutation-independent mechanisms that affect mycobacterial susceptibility to macrolides. To address this objective, this project is divided in to 3 specific aims: (1) to identify the genes conferring inducible macrolide resistance; (2) to investigate the macrolide efflux systems of mycobacteria; and, (3) to characterize the prevalence of resistance genes within the Mycobacteriaceae. Understanding the processes that affect the antimycobacterial activity of macrolides will directly impact the development of new drugs and improve treatment regimens. Of particular interest is improving the anti-tuberculosis activity of macrolides.

Grant: 1R01AI052293-01A1
Program Director: PERDUE, SAMUEL S.
Principal Investigator: ZGURSKAYA, HELEN I PHD
Title: Transport across two membranes by AcrAB-ToIC complex
Institution: UNIVERSITY OF OKLAHOMA NORMAN NORMAN, OK
Project Period: 2003/03/01-2008/02/29

DESCRIPTION (provided by applicant): Three-component protein complexes spanning two membranes are universally spread among Gram-negative bacteria and have been implicated in such diverse range of transport functions as delivery of virulence factors into the hosts, secretion of signaling molecules and protection of bacterial cells against structurally diverse antimicrobial agents. A remarkable feature of these transporters is that the substrate transfer occurs across two membranes directly into external medium bypassing the periplasmic space. Special periplasmic proteins, which belong to the Membrane Fusion Protein (MFP) family, are responsible for the coupling of two membranes. The major objective of this project will be to investigate the mechanism of coupling of two membranes using the multidrug efflux transporter AcrAB-TolC from *E. coli* as a model complex. The AcrAB-TolC extrudes out of the cell a broad range of antimicrobial compounds including antibiotics, detergents, dyes and organic solvents. Located in the inner membrane AcrB transporter captures its substrates within phospholipid bilayer of inner membrane and transports them into external medium via the outer membrane channel, TolC. The cooperation between AcrB and TolC is mediated by the MFP protein, AcrA. Biochemical studies suggested that AcrA might coordinate the function of the complex by bringing the inner and outer membranes into proximity. The applicants intend to test this hypothesis critically and to investigate the mechanism of AcrA. The specific aims are: (1) investigate if AcrA has differential affinity for the inner and outer membranes; (2) using limited proteolysis and chemical cross-linking, characterize the conformation of AcrA in vivo and in vitro; (3) characterize conformational transitions of AcrA in vitro; and (4) investigate physical and functional interactions between AcrA, AcrB and TolC in vitro. These studies are expected to yield a comprehensive picture of AcrA-mediated coupling of two membranes. These data will contribute to an understanding of the mechanism of multidrug efflux in Gram-negative bacteria and will illuminate the role of MFP proteins in the coordination of transport across two membranes.

Grant: 1R01AI052330-01A1
Program Director: PERDUE, SAMUEL S.
Principal Investigator: REYNOLDS, KEVIN A PHD
Title: 3-Ketoacyl ACP Synthase III: A Novel Antibiotic Target
Institution: VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA
Project Period: 2003/03/01-2008/02/29

DESCRIPTION (provided by applicant): Fatty acid biosynthesis by a type II dissociated fatty acid synthase (FAS) is a fundamental and indispensable metabolic pathway in many organisms, including bacteria and parasites. The distinctions between this and the multifunctional type I FAS of higher organisms offer a selective target for the design of novel antibacterial and antiparasitic agents, needed to combat the resurgence of antibiotic-resistant bacteria and multidrug-resistant forms of the malaria-causing plasmodial parasites. In all type II FAS systems, the initial condensation step is carried out by 3-ketoacyl acyl carrier protein (ACP) synthase III (KASIII, FabH), which catalyzes the condensation of an acyl CoA substrate with malonyl ACP to generate a 3-ketoacyl ACP product. FabH also appears to play a key role in regulation of fatty acid biosynthesis and is not targeted by any current drugs, making it a particularly attractive new target for drug design. The long-term objective of our work is to understand the structural and mechanistic bases of FabH that define its physiological roles and to use this information to generate novel potent and selective inhibitors. This grant will extend our study of FabH enzymes from organisms such as *Escherichia coli* and *Staphylococcus aureus*, which initiate *de novo* fatty acid biosynthesis from different short chain acyl CoA substrates, and *Mycobacterium tuberculosis*, which uses FabH to initiate mycolate biosynthesis from long chain acyl CoA substrates. Mutational analyses and crystallography will be used to investigate the differing substrate and inhibitor specificities of these enzymes. In conjunction with ongoing crystallographic, molecular modeling and kinetic analyses, the mode of binding of 1,2-dithiole-3-one and related compounds which are potent novel active site FabH inhibitors, and a second series of inhibitors that appear to bind in the FabH phosphopantetheine binding channel, will be investigated. The information gathered from these studies will be used to design, synthesize and ultimately test inhibitors, which maximize interactions with both active site residues and those in either the acyl-binding pocket or phosphopantetheine-binding channel. Such compounds should have enhanced activity and selectivity against FabH and be powerful new lead compounds for development of novel antibacterial and antiparasitic/antimalarial drugs.

Grant: 1R01AI052354-01A1
Program Director: VAN DE VERG, LILLIAN L.
Principal Investigator: SNAPPER, SCOTT B
Title: Cytoskeletal-Pathogen Interactions in Shigella Infection
Institution: MASSACHUSETTS GENERAL HOSPITAL BOSOTN, MA
Project Period: 2003/09/15-2007/12/31

DESCRIPTION (provided by applicant): Shigella are gram negative enteric pathogens that cause severe diarrheal disease and have been classified as a Category B Biological Agent. Shigella pathogenesis requires bacterial invasion of the colonic epithelium and bacterial spread through the colonic mucosa. Shigella entry into epithelial cells is mediated by effector molecules, secreted through a type III secretion apparatus, that activate Rho family GTPase signaling pathways to induce the formation of cell surface projections and membrane ruffles that engulf the bacteria by macropinocytosis. Both Cdc42 and Rac have been implicated as having a role in the Shigella entry process. Cdc42 is known to activate Rac; it is not clear whether Cdc42 involvement in Shigella entry is mediated exclusively via this link. Moreover, the downstream effectors of Cdc42 and/or Rac activation during Shigella entry are unknown. We have recently confirmed that the major Shigella pathway is Cdc42-dependent. However, we have also demonstrated the existence of a novel Cdc42-independent invasion pathway. Furthermore we have shown that the only known downstream effector of Cdc42 that activates the actin cytoskeleton, N-WASP, is not involved in Shigella entry. Once in the cytoplasm, Shigella moves by active assembly of an actin tail. Actin tail formation is mediated by the Shigella outer membrane protein IcsA, which binds and activates N-WASP. Activated N-WASP stimulates Arp2/3 complex-mediated actin assembly. The molecular mechanism by which IcsA binds and activates N-WASP is poorly understood. Our goals in this proposal are to: 1. Define the specific roles of Cdc42 and Rac in Shigella entry; 2. Identify and characterize the downstream effectors of Rho family activation during Shigella entry; and, 3. Elucidate the mechanism(s) by which Shigella IcsA activates N-WASP and determine whether this mechanism mimics Cdc42 activation of N-WASP. These studies will define the specific cellular signaling pathways required for Shigella entry and actin tail formation and will identify downstream pathways of Rho family activation.

Grant: 1R01AI052473-01A1
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: KO, ALBERT I BS
Title: Natural History of Leptospirosis
Institution: WEILL MEDICAL COLLEGE OF CORNELL NEW YORK, NY
UNIV
Project Period: 2003/02/15-2008/01/31

DESCRIPTION (provided by applicant): Leptospirosis, a zoonotic spirochaetal disease with worldwide distribution, is an emerging infectious disease. We have shown that leptospirosis has now spread from its traditional rural base to become the cause of cyclic rainfall-associated epidemics in the urban setting. Conditions of climate and growing urban poverty have contributed to the emergence of this new epidemiological pattern: large outbreaks associated with high mortality occur each year during the same seasonal period and affect the same risk groups within urban slum communities. The public health priority in response to these epidemics is to address the severe clinical forms, such as Weil's disease and severe pulmonary haemorrhage syndrome, for which mortality is >15 percent. Current treatment and control measures have not made an impact in reducing this mortality and therefore, new preventative approaches need to be developed. However, little is known regarding why a small proportion (5-15 percent) of individuals progress to develop severe clinical outcomes. We hypothesize that that in high transmission settings, severe clinical outcomes are influenced by differences in the inoculum size during exposure to environmental factors and by acquired immune response after natural infection. In the city of Salvador, Brazil, we have established the epidemiological and laboratory infrastructure to study leptospirosis. Five-year surveillance at this site has identified more than 1400 severe leptospirosis cases, therefore providing a unique opportunity to study the natural history of leptospirosis. We propose a community-based longitudinal study that aims to: (1) Determine whether environmental risk exposures, including those that may influence inoculum size during infection, are associated with an increased risk of developing severe disease after infection, and (2) Determine prospectively whether immunological responses, acquired during a prior infection, protect against re-infection with pathogenic *Leptospira*. These studies should identify potential preventative measures for severe leptospirosis. At the same time, they may provide more widely applicable benefits such as the development of improved diagnostic tools and identification of targets for vaccine development.

Grant: 1R01AI052813-01A1
Program Director: LAMBROS, CHRIS
Principal Investigator: KOEHLER, JANE E MD
Title: MECHANISMS OF BARTONELLA VIRULENCE IN AIDS PATIENTS
Institution: UNIVERSITY OF CALIFORNIA SAN FRANCISCO, CA
FRANCISCO
Project Period: 2002/12/15-2007/11/30

(Revised Abstract) The long range objective of the proposed study is to gain insight into the pathogenic mechanisms of Bartonella, an opportunistic pathogen of AIDS patients. *B. henselae* and *B. quintana* are fastidious, gram-negative bacteria that cause bacillary angiomatosis (BA), a vascular proliferative lesion affecting HIV-infected patients. Relapsing and/or persistent bloodstream infection is a frequent manifestation of *B. quintana* infection that occurs in patients at all stages of HIV infection and can last for months in humans. Causing debilitating and even fatal sequelae. We recently identified a protein of Bartonella that has a number of characteristics in common with other virulence determinants of bacterial pathogens that are able to successfully and persistently infect the human host. This protein appears to be involved in Bartonella pathogenesis in humans. The immediate objective of this proposal is to study the mechanisms of Bartonella pathogenesis by elucidating the virulence properties of the *B. quintana* protein, including characterization of the interactions with the host in vitro and in vivo, and the clinical and molecular correlation of expression of this protein in isolates from AIDS patients. The ultimate goal of this project is to identify the contribution of this protein to Bartonella-mediated pathogenesis in HIV-infected patients at the bacterial and host molecular and cellular levels.

Grant: 1R01AI053067-01A1
Program Director: SCHMITT, CLARE K.
Principal Investigator: SPERANDIO, VANESSA PHD
Title: Quorum sensing regulation of EHEC virulence genes
Institution: UNIVERSITY OF TEXAS SW MED DALLAS, TX
CTR/DALLAS
Project Period: 2003/07/01-2007/12/31

DESCRIPTION (provided by applicant): Enterohemorrhagic *E. coli* (EHEC) O157:H7 causes bloody diarrhea and hemolytic uremic syndrome (HUS) throughout the world. EHEC has a very low infectious dose, making it difficult to control epidemiologically. EHEC colonizes the large intestine where it causes attaching and effacing (AE) lesions, and also produces Shiga toxins (Stx) that are responsible for the major symptoms of HUS. Although the virulence mechanisms of EHEC have been extensively studied, very little is known about how EHEC regulates the expression of its virulence genes. We recently reported that both of the genes involved in AE lesion formation, as well as Stx production, are activated by quorum sensing (QS). QS is a cell-to-cell signaling mechanism in which bacteria secrete hormone-like compounds (autoinducers) that interact with bacterial transcriptional regulators to drive gene expression. The QS mechanism employed in this activation is primarily involved in bacterial inter-species communication, and we propose that activation of EHEC virulence genes by this system would occur in response to autoinducers produced by the normal intestinal flora (which could be an explanation for its low infectious dose). This proposal represents a comprehensive effort to study virulence gene regulation in EHEC. In Specific Aim 1, we shall identify other previously uncharacterized EHEC-specific QS regulated genes. Given that QS regulation is critical for EHEC pathogenesis, the identification of such genes expressed in concert with known virulence factors may lead to the discovery of novel virulence genes. In Specific Aim 2, we shall identify and characterize the transcriptional regulators of the QS regulon. Specific Aim 3 is designed to address the role of QS not only in bacterial-bacterial but also bacterial-host cell communication, and monitor QS virulence gene regulation "in vivo". The proposed experimental approaches will achieve a better understanding of the sensory events by which EHEC responds to activate its virulence genes, and may lead to the identification of other virulence genes and novel targets for the potential development of more effective intervention strategies for EHEC disease.

Grant: 1R01AI053075-01A1
Program Director: KLEIN, DAVID L
Principal Investigator: HARVILL, ERIC T BS
Title: Comparative Immunobiology of the bordetellae
Institution: PENNSYLVANIA STATE UNIVERSITY-UNIV UNIVERSITY PARK, PA
PARK
Project Period: 2003/09/15-2007/12/31

DESCRIPTION (provided by applicant): The genus *Bordetella* includes important human and animal respiratory pathogens. Despite its extensive use since the 1940s, the *Bordetella pertussis* vaccine has substantial problems; it provides incomplete protection from disease and has no effect on the infection rate, allowing bacteria to circulate on an ongoing basis. Although it is clear that this intramuscular vaccine does not generate the same protection as infection of the respiratory mucosa, lack of understanding of the mechanisms of protective immunity hampers its improvement. In the mouse model, B cells are required to clear infection but adoptive transfer of immune serum had little effect on bacterial numbers, interpreted to mean B cells perform some function other than antibody production. We propose an alternative interpretation; secretory antibodies are the primary mechanism of protective immunity to *B. pertussis*. This would explain the requirement for B cells and the lack of effect of transfer of immune serum, which contains little IgA. It also suggests that vaccines prevent severe disease via serum antibody-mediated neutralization of toxins, but do not affect bacterial colonization levels because they do not induce secretory IgA within the respiratory tract. We have recently observed that, unlike *B. pertussis*, serum antibodies are sufficient to clear *B. bronchiseptica* from the lower respiratory tract of mice. The extraordinarily close phylogenetic relatedness and the genomic sequences of these two subspecies will allow both similarities and differences to be both related to the diseases they cause and interpreted in light of their comparative genomics and the recent emergence of the human pathogen. In this proposal we will determine the roles of antibodies, and their mechanisms of action, in control and clearance of *Bordetella* subspecies. Aim 1. We will use mice lacking B cells and adoptive transfer of antibodies, and specific isotypes, to determine the role of antibodies in bacterial control/clearance. Aim 2. Mice lacking specific antibody effector functions will be used to determine the contribution of each to the antibody effects. Preliminary results indicate these effects, and the mechanisms involved, differ in various respiratory organs and with different *Bordetella* subspecies. Aim 3. We have determined that adoptively transferred antibodies have very different effects than do those endogenously produced in response to infection, paralleling observations of vaccine-induced, as opposed to infection-induced, immunity. We will compare adoptively transferred and naturally produced antibodies using wild type, B cell deficient or IgA-deficient mice to understand the basis for these differences. These experiments will characterize the interactions of *Bordetella* subspecies with host antibodies to determine the mechanisms of antibody-mediated bacterial clearance from the respiratory tract. Similar mechanisms are likely to be involved in respiratory immunity to other organisms, including those considered potential bioterrorism threats.

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Grant: 1R01AI053101-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: STEVENSON, BRIAN PHD
Title: *Borrelia burgdorferi* LuxS-mediated quorum sensing
Institution: UNIVERSITY OF KENTUCKY LEXINGTON, KY
Project Period: 2003/06/15-2007/11/30

DESCRIPTION (provided by applicant): The spirochete *Borrelia burgdorferi*, the causative agent of Lyme disease, is transmitted to humans and other warm-blooded animals through the bite of infected *Ixodes* spp. ticks. The establishment of *B. burgdorferi* infection involves numerous interactions between the bacteria and a variety of vertebrate host and arthropod vector tissues. Different bacterial proteins are required at specific points of this infectious cycle, and precise regulation of the synthesis of such proteins is essential for successful infection to occur. We have discovered that these spirochetes utilize a regulatory mechanism to control protein expression patterns that involves a chemical signal known as autoinducer-2 (AI-2). This molecule is produced by the *B. burgdorferi* LuxS protein, which we have demonstrated to be a functional enzyme. Our preliminary studies suggest that *B. burgdorferi* can regulate LuxS synthesis. Addition of AI-2 to cultured *B. burgdorferi* dramatically alters the expression of more than 50 different proteins, increasing expression levels of some proteins, while decreasing expression of others. Through this mechanism, a population of Lyme disease spirochetes may synchronize production of proteins needed for infection processes. We hypothesize that *B. burgdorferi* uses AI-2 as an important signaling molecule to control expression of proteins during the natural infectious cycle of the Lyme disease spirochetes. The proposed studies will characterize the expression of LuxS during bacterial infection, the mechanisms by which *B. burgdorferi* controls synthesis of AI-2, and functions of AI-2-regulated proteins. Results from these studies will enhance our understanding of the complex interactions between *B. burgdorferi* and its hosts, as well as identifying potential bacterial targets for improved therapies to prevent and treat Lyme disease.

Grant: 1R01AI053109-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: OSTFELD, RICHARD S PHD
Title: Biodiversity, Habitat Fragmentation, & Lyme Disease Risk
Institution: INSTITUTE OF ECOSYSTEM STUDIES MILLBROOK, NY
Project Period: 2003/01/15-2006/12/31

DESCRIPTION (provided by the applicant): We propose a research program that focuses on the interactions among tick vectors, bacterial pathogens, the community of vertebrate hosts, and the ecological landscapes in which vectors, pathogens, hosts, and humans interact to affect exposure to Lyme disease. We focus on Lyme disease because of its high incidence, widespread distribution, large body of ecological and epidemiological background information, and potential as a model of other vector-borne zoonoses. Our research over the past 9 years has generated a conceptual model that we call the dilution effect. According to this model, high species diversity in the community of hosts for ticks reduces the infection prevalence of ticks by diluting the effects of the ubiquitous white-footed mouse, which is the principal natural reservoir for the disease agent, *Borrelia burgdorferi*. Other published research suggests that species diversity of vertebrate's decreases, and population density of mice increases, with decreasing forest patch size. We propose to test this model rigorously at sites in six northeastern states. Our research will focus on the generality of the dilution effect and on the mechanisms that underlie it. We will assess the species richness and relative abundance of vertebrate hosts, as a function of size of forest fragments, in suburban landscapes of the Northeast. We will use both species diversity indices and patch size as independent variables in regressions that use either infection prevalence of nymphal ticks, or the abundance of infected nymphs, as dependent variables. Finally, we will use modeling approaches developed in our lab to both assess the generality of the dilution effect for other vector-borne diseases and increase the realism of the Lyme-disease system. Our research comprises a rigorous, multidisciplinary approach to understanding the linkages between ecology and risk of infectious disease.

Grant: 1R01AI053111-01A1
Program Director: PERDUE, SAMUEL S.
Principal Investigator: MINNICK, MICHAEL F PHD
Title: Hemin Receptor Gene Family of Bartonella quintana
Institution: UNIVERSITY OF MONTANA MISSOULA, MT
Project Period: 2003/06/15-2006/11/30

DESCRIPTION (provided by applicant): Five Bartonella species are emerging infectious agents responsible for Oroya fever, cat-scratch disease, bacillary angiomatosis, and trench fever in humans. Life-threatening complications of bartonellosis can include endocarditis, peliosis hepatis, relapsing bacteremia, encephelopathy and neuroretinitis. Bartonella quintana, the model for this study, is currently re-emerging in inner-city homeless people and in patients suffering from AIDS. Like all Bartonella, B. quintana colonizes the circulatory system, where it infects human erythrocytes, vascular endothelial cells, triggers angiogenesis and causes persistent bacteremia. Despite these remarkable attributes, little is known about the molecular pathogenesis of Bartonella. Because hemin is an essential growth factor for all Bartonella species and B. quintana has the greatest hemin requirement known for any bacterium, the long range goal of this study is to examine the molecular basis for hemin acquisition-- a process that would contribute not only to establishment of infection but persistence in the arthropod vector and human host. To this end, the proposal focuses on analysis of a five-gene family encoding B. quintana's major hemin receptor and four homologues. Specific Aim 1 will quantify hbp expression in response to varying hemin concentration using RT-PCR. We will also analyze the potential ferric uptake regulator (fur) box using electrophoretic mobility shift assays and DNA footprinting, and we will map hbp transcription initiation sites. We will also verify Fur's role in hbp regulation by quantifying hbp expression in both fur mutant and over-expressed fur backgrounds. In Specific Aim 2 we will analyze the expression patterns of the hbp multigene family over the course of infection in the human louse vector and a macaque primate model. In addition, a mutant for the dominant hbp gene and a trans-complemented strain will be generated to test molecular Koch's postulates in the primate model. In Specific Aim 3, we will determine the structure and function of the Hbp proteins by mapping functional receptor domains using biochemical and genetic approaches. In addition, we will determine whether Hbp's can transport hemin and will identify domains that are necessary for this function. These data will provide valuable information on a multigene family involved in an essential process for Bartonella growth and persistence. Further, since Bartonella Hbp's are possibly members of an outer membrane protein family from several Gram-negative bacteria, data generated from this study will undoubtedly be of broad importance to bacterial pathogenesis.

Grant: 1R01AI053212-01
Program Director: KORPELA, JUKKA K.
Principal Investigator: ABEL-SANTOS, ERNESTO V PHD
Title: Antimicrobial targets of intracellular cyclic peptides
Institution: YESHIVA UNIVERSITY BRONX, NY
Project Period: 2003/01/01-2007/12/31

DESCRIPTION (provided by applicant): The emergence of multidrug resistant infections coupled with the continuous threat of biological warfare has created the need for the discovery of new antimicrobial targets. This project will utilize a novel genetic strategy to functionally interrogate microbial genomes for gene products required for cell viability. Making the connection between gene sequence and protein essentiality is ever so crucial since 30 to 50 percent of microbial ORFs have no assigned biological function. E. coli will serve as a paradigm for enteric infections, since strains have been engineered with very high transformation efficiencies. The main objective of this proposal is to detect essential bacterial genes, while simultaneously obtaining stable, cyclic peptides that inhibit their function(s). The specific aims of this project are: (1) Identification of the cellular targets for individual toxic peptides, (2) Selection of potential antimicrobial targets and analysis of the structural and chemical determinants required for their inhibition, and (3) Genome-wide detection of metabolic functions required for cell viability. The results of our efforts will provide new targets and lead compounds for antibiotic design. The research plan will require the production and selection of vast numbers of cyclic peptide sequences in E. coli. These libraries will be obtained using the Split Intein Circular Ligation Of Peptides and ProteinS SICLOPPS) technology. The experimental method will involve screening for bactericidal/bacteriostatic peptides using replica plating and/or flow cytometry. Each identified toxic peptide will be co-transformed with every ORF found in the host genome. The obstructed metabolic function(s) will be restored by over-expression of the targeted protein(s). The genomic constructs encoding essential, peptide-inhibited functions will be recovered by PCR and their identity revealed by sequencing or by hybridizing onto DNA arrays. Essential genes conserved across bacterial species and not represented in humans will be selected for further studies. For each potential target selected, the intracellular peptide library will be re-screened to detect all inhibitors of the isolated biological function. The family of retrieved peptides will be aligned to identify conserved amino acids required for inhibition.

Grant: 1R01AI053264-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: MADAN, ANUP PHD
Title: Genetic Mechanisms Driving Evolution of Rickettsiaceae
Institution: INSTITUTE FOR SYSTEMS BIOLOGY SEATTLE, WA
Project Period: 2003/01/15-2003/07/31

DESCRIPTION (provided by applicant): The overall goal of the proposed research is to significantly enhance our present understanding of the properties of rickettsiae, a group of obligately intracellular, arthropod transmitted, bacteria which cause at least eleven human diseases in different parts of the world. *Orientia tsutsugamushi*, *Rickettsia akari*, *R. canada*, and *R. bellii* will be studied. The complete genome sequences of these organisms will be determined to 99.99% accuracy and analyzed by computer to identify the putative RNA, protein encoding genes, pseudogenes, repeat elements, 'selfish DNA', and regulatory elements present in each organism. This information will be provided in publicly accessible databases. Unique and conserved genetic elements found in each rickettsia will be compared to help understand the mechanisms underlying evolution of their pathogenic adaptations. This initial in silico analysis requires biochemical confirmation as it depends upon the similarity of rickettsial gene sequences to well-characterized genetic elements found in other bacteria. Less than 15 of the 1000-2000 genes expected to be present in each of these species have been studied previously. Because of the intracellular lifestyle of rickettsiae and the lack of genetic systems, conventional approaches to characterization of the functional properties of these genes in rickettsiae are limited. Bacterial artificial chromosome (BAC) libraries and DNA arrays containing each gene will be constructed for each rickettsial genome. The BACs and DNA arrays will be used to characterize the expression and regulation of rickettsial genes. Selected rickettsial proteins that may be useful in vaccine development and as potential therapeutic targets will be cloned and expressed to facilitate their characterization.

Grant: 1R01AI053279-01A1

Program Director: BAKER, PHILLIP J.

Principal Investigator: FLAVELL, RICHARD A PHD
BIOCHEMISTRY:PROTEIN/A
INO ACID

Title: Vaccine responsiveness to *Borrelia burgdorferi* OspA

Institution: YALE UNIVERSITY NEW HAVEN, CT

Project Period: 2003/07/01-2007/12/31

DESCRIPTION (provided by applicant): The Lyme disease vaccine is based on OspA, a *Borrelia burgdorferi* lipoprotein, and immunity is contingent upon high levels of OspA antibodies. Toll-like receptors (TLRs) are important for the initiation of immune responses to pathogens and TLR2 recognizes bacterial lipoproteins, including OspA. Our preliminary data now demonstrate that TLR1 is also involved in recognizing OspA. Therefore defects in TLR-mediated signaling could result in ineffective lipoprotein-recognition and influence responsiveness to OspA-vaccination. In this proposal we will explore the association between TLR1, TLR2, and responsiveness to vaccination with OspA in mice and humans. We have now identified 7 persons with very low OspA antibody titers after vaccination. Macrophages from these individuals produced less TNF- α after stimulation with OspA - but not peptidoglycan - than controls; suggesting a defect in signaling that is partially associated with TLR2 (because peptidoglycan is also recognized by TLR2). In vitro transfection studies then demonstrated that dominant-negative TLR1 could inhibit TLR2-mediated OspA responsiveness, implying that TLR1 and TLR2 cooperate for lipoprotein recognition. This was further examined in Tlr2^{-/-} mice, and Tlr1^{-/-} mice that we have created. Tlr1^{-/-} or Tlr2^{-/-} mice made lower levels of OspA antibodies, after immunization with OspA, than controls. Macrophages from Tlr2^{-/-} mice responded poorly to both OspA and peptidoglycan whereas cells from Tlr1^{-/-} mice responded to peptidoglycan - but not OspA. These preliminary data suggest that TLR1 and TLR2 are required for OspA recognition, that the absence of TLR1 or TLR2 results in impair antibody responses to OspA immunization in mice, and that defects in the TLR1/2 signaling pathway may account for human hyporesponsiveness to OspA vaccination. We will now fully characterize the response to OspA in Tlr1^{-/-} or Tlr2^{-/-} mice, and in humans that do not develop significant OspA antibodies following vaccination. These studies may provide an understanding of the molecular mechanisms for variability in antibody responses to the OspA vaccine, a first glimpse into TLR deficiencies in humans, and lead to new approaches to augment immune responses to OspA and other vaccines.

Grant: 1R01AI053417-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: GLICKMAN, MICHAEL S MD
Title: Cyclopropane Synthetases and M. tuberculosis Virulence
Institution: SLOAN-KETTERING INSTITUTE FOR NEW YORK, NY
CANCER RES
Project Period: 2003/07/01-2007/12/31

DESCRIPTION (provided by applicant): Mycobacterium tuberculosis (Mtb) infects one third of the world's population and kills approximately 2 million people per year worldwide. Despite over a century of investigation, the molecular mechanisms of Mtb pathogenesis are still largely unknown. We are investigating the role of the Mtb cell envelope in pathogenesis through the creation and characterization Mtb mutants. Through this approach, we have established that two members of a novel gene family in Mtb, the mycolic acid cyclopropane synthetases, modify the major lipids of the Mtb cell envelope with specific stereochemistries of cyclopropyl residues. In addition, we have established that deletion of individual cyclopropane synthetases has dramatic effects on the pathogenesis of Mtb infection in mice, including alterations in the host immune response and consequent tissue destruction. To further study the role of mycolic acid cyclopropanation in Mtb pathogenesis, we have generated a set of cyclopropane synthetase mutants through allelic exchange. We will define the function of each these genes in mycolic acid modification through the examination of mycolic acid profiles in the mutant strains. Furthermore, we will define the pathogenetic role of each synthetase in mouse models of Mtb infection. We will study the mechanism by which cyclopropanated mycolic acids affect pathogenesis by characterizing the Trehalose Dimycolate (TDM) from the mutant strains and examining the role of innate immunity in the recognition of cyclopropane deficient mycolic acids. These studies will elucidate the role of mycolic acid cyclopropane modification in Mtb pathogenesis and examine the mechanism by which the fine chemical structure of the Mtb mycolic acids mediates symbiosis in vivo. These studies will contribute to our long-term goal of understanding the relationship between the chemical complexity of the Mtb cell envelope and the pathogenesis of Mtb infection. This knowledge may validate this new antibiotic target in Mtb and lead to new vaccine strategies.

Grant: 1R01AI053422-01A1
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: KLIMPEL, GARY R PHD
Title: Human Cellular Immune Responses to Leptospira
Institution: UNIVERSITY OF TEXAS MEDICAL BR GALVESTON, TX
GALVESTON
Project Period: 2003/09/30-2008/01/31

DESCRIPTION (provided by applicant): Leptospirosis, a zoonotic disease caused by spirochetes of the genus *Leptospira*, is transmitted to humans via urine of infected mammals. Found worldwide in temperate and tropical climates, it is a public health threat in the U.S. and abroad. Human infections by *Leptospira* have a broad spectrum of clinical manifestations, ranging from asymptomatic seroconversion to undifferentiated febrile syndrome, with or without aseptic meningitis, to fulminant jaundice, renal failure and hemorrhage with a fatality rate up to approximately 25%. There is no vaccine to prevent human leptospirosis and veterinary vaccines may not be used in humans because of toxicity. Little is known about how this organism interacts with human lymphocytes and/or the mechanisms of leptospiral pathogenesis or immunity. We propose to examine molecular and cellular interactions of *Leptospira* with human peripheral blood mononuclear cells (PBMC) with a focus on different T cell populations. We have found that leptospires activate T cells in PBMC from *Leptospira*-naïve humans, leading to proliferation and production of high IFN gamma levels. Our hypothesis is that *Leptospira* activation of T cells expressing the alpha/beta or gamma/delta form of the T cell receptor for antigen plays an important regulatory role in protective immunity and immunopathogenesis associated with human infections due to *Leptospira* spp. Our aims are to: 1) Determine the cell:cell interactions and cytokine profiles of PBMC cultures exposed to *Leptospira*, and characterize the phenotype, function, and specificity of T cells activated in such cultures; 2) Identify and purify *Leptospira* components that activate T cells; and 3) Determine the relationship between peripheral blood T cell phenotype and function and disease severity of human leptospirosis patients in an endemic region in the Peruvian Amazon. Learning how *Leptospira* interact with the human immune system will lead us to understand the pathogenesis of leptospirosis and how to develop vaccines and strategies to control the associated pathology.

Grant: 1R01AI053593-01
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: ELKINS, CHRISTOPHER PHD
Title: Vaccine Studies of the hemoglobin receptor of *H. ducreyi*
Institution: UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC
HILL
Project Period: 2003/02/01-2006/01/31

DESCRIPTION (provided by applicant): *Haemophilus ducreyi*, the etiologic agent of the genital ulcer disease chancroid, is a significant public health problem in several regions worldwide. In Africa, Asia and other developing countries, it is an important cofactor for the heterosexual transmission of HIV. Control of chancroid, using an effective vaccine that is properly administered, would likely reduce HIV transmission. It is the goal of my laboratory to develop such a vaccine. We propose studies on the immunobiology and structure of the hemoglobin receptor (HgbA) of *H. ducreyi*. Several attributes of HgbA make it an attractive vaccine candidate. HgbA is required to establish human experimental infection. HgbA is conserved immunologically and functionally and all *H. ducreyi* express it. Unlike hemoglobin receptors from other bacteria, HgbA does not undergo phase or antigenic variation. Studies in the chilled rabbit model of chancroid infection, suggest purified native HgbA or recombinantly expressed HgbA are partially effective vaccines. Further vaccine studies utilizing the Swine model of *H. ducreyi* infection, that more closely resembles natural human infection. Experimental vaccines will consist of purified native HgbA from *H. ducreyi* type strain 35000, or possibly synthetic peptides derived from HgbA. We will test the ability of native HgbA to protect against a homologous and heterologous challenge infection. Detailed studies will be undertaken on HgbA including understanding its structure and regions/residues of HgbA that are surface-exposed. We will determine the variability of the HgbA protein from geographically diverse isolates. These studies are important for better understanding of chancroid pathogenesis and will facilitate vaccine development in several aspects.

Grant: 1R01AI053669-01A1
Program Director: HALL, ROBERT H.
Principal Investigator: HIGGINS, DARREN E PHD
Title: Temporal Requirements for Intracellular Pathogenesis
Institution: HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA
Project Period: 2003/07/01-2007/12/31

DESCRIPTION (provided by applicant): *Listeria monocytogenes* (L.m.) is an intracellular bacterial pathogen that causes serious food-borne illness in pregnant women, the elderly and immunocompromised individuals. Listeriolysin O (LLO), a pore-forming cytolysin, and two bacterial phospholipases, PI-PLC and PC-PLC, are essential determinants of pathogenesis that mediate lysis of host cell vacuoles resulting from bacterial entry and intracellular spread. LLO is also expressed during intracytosolic growth and mediates numerous alterations in host cell physiology. During in vitro infection of cell lines, LLO is sufficient to facilitate lysis of all vacuolar membranes. Yet, PI-PLC and PC-PLC increase the efficiency of membrane lysis. It is hypothesized that these determinants play a specific role in the dissolution of each vacuolar membrane and the intracytosolic production of LLO is necessary for optimal intracellular growth. The focus of this proposal is to precisely define the temporal requirement of LLO for intracellular growth and cell-to-cell spread in primary host cells and for the maintenance of in vivo infection in a mouse infection model. In Aim I, the precise requirement for LLO expression during intracellular growth and spread in primary host cells will be determined. This will be accomplished by using a novel genetic approach to allow regulated production of LLO during intracellular infection. Intracellular LLO levels will be varied during infection of primary host cells and bacterial replication and spread determined by microscopic analysis and enumeration of intracellular bacteria. In Aim II, the precise roles of LLO, PI-PLC and PC-PLC in dissolution of vacuolar membranes during intracellular spread will be identified. L.m. strains allowing regulated expression of LLO in PI-PLC and PC-PLC mutants will be used in mixed host cell infections. Plaque formation in cell monolayers, differential time-lapse fluorescence microscopy and high-resolution electron microscopy will be used to evaluate progression of infection and dissolution of vacuolar membranes. In Aim III, we will evaluate the requirement of LLO expression for maintenance of in vivo infection and the development of acquired immunity. BALB/c mice will be infected under varying times of in vivo LLO induction. Progression of infection will be evaluated by enumerating bacteria from organs and comparing to infection of wild-type and defined L.m. mutants. Immunological assessment will be determined by ELISPOT analysis and protection from wild-type bacterial challenge.

Grant: 1R01AI053674-01A1
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: HAUSER, ALAN R MD
Title: Bacterial Predictors of Severe Nosocomial Pneumonia
Institution: NORTHWESTERN UNIVERSITY EVANSTON, IL
Project Period: 2003/07/01-2007/12/31

DESCRIPTION (provided by applicant): The long-term objective of the proposed study is to better understand the pathogenesis of hospital-acquired pneumonia caused by *Pseudomonas aeruginosa*. The general strategy is to characterize the role of the *P. aeruginosa* type III secretion system in hospital-acquired pneumonia. This complex secretion pathway transports and injects four known effector proteins into host cells: ExoS, ExoT, ExoU (also known as PepA), and ExoY. Interestingly, clinical isolates differ in the combination of effector proteins they secrete. Recent studies are beginning to define the role of individual effector proteins in pathogenesis. Preliminary studies using bacterial mutants indicate that ExoS, ExoT, ExoU, and ExoY all have cytotoxic effects on mammalian cells in cell culture systems. ExoT, ExoU, and possibly ExoS contribute to virulence in animal models of pneumonia. In addition, ExoU secretion is associated with worse clinical outcomes in humans with hospital-acquired pneumonia. Together, these findings support an important role for type III effector proteins in the acute pneumonia, although the exact role of each effector protein and the mechanisms by which these proteins lead to the pathophysiological consequences of pneumonia remain to be defined and are the subject of this proposal. Our preliminary data suggest that type III secretion contributes to bacterial persistence, dissemination, and mortality as well as neutrophil killing and suppression of proinflammatory cytokine release in a mouse model of pneumonia. Further defining the role of individual effector proteins in these processes is crucial to our understanding of the pathogenesis of hospital-acquired pneumonia caused by *P. aeruginosa*. It is hypothesized that specific effector proteins play an important role in the pathogenesis of hospital-acquired pneumonia, including the prevention of bacterial clearance by neutrophils. Furthermore, it is hypothesized that because of these effects secretion of specific effector proteins can be used as markers for strains associated with especially severe hospital-acquired pneumonia in human patients. Studies using both mice and humans will be performed to define the roles of these effector proteins in the pathogenesis of acute pneumonia, including modulation of the inflammatory response and resistance to neutrophil-mediated clearance, and to determine whether secretion of particular effector proteins serves as a marker for strains capable of causing especially severe disease in human patients with hospital-acquired pneumonia.

Grant: 1R01AI053692-01A1
Program Director: PERDUE, SAMUEL S.
Principal Investigator: BROWN, WENDY C PHD
Title: Identification of T-Cell Immunogens in Anaplasma
Institution: WASHINGTON STATE UNIVERSITY PULLMAN, WA
Project Period: 2003/08/01-2008/01/31

DESCRIPTION (provided by applicant): MHC class II-restricted responses mediated by CD4+ T lymphocytes are central to immune control of the tick-borne pathogens of the Family Anaplasmataceae. Priming and expansion of CD4+ T lymphocytes is required for development of high affinity neutralizing IgG antibodies directed against the bacterial surface and for efficient phagocyte activation leading to bacterial killing. The goal of the research is to identify the pathogen proteins that induce the T cell responses required for immunity. To date, only four T cell immunostimulatory molecules have been identified in *Anaplasma marginale*, the type species, and none in the related human pathogens within the Anaplasmataceae. We will address this knowledge gap using a comprehensive strategy to identify outer membrane proteins that prime naive CD4+ T lymphocytes and induce recall memory T cell responses of immunized and protected animals. In part 1 of the project, we propose to use a combination of biochemical fractionation of outer membranes combined with genomic analysis to identify the targets of the T cell response. At the conclusion of part 1, we expect to have identified novel outer membrane proteins bearing CD4+ T cell epitopes conserved among *A. marginale* strains. The presence of molecular and immunological orthologs among species in the Family Anaplasmataceae indicates that the research results should be broadly applicable and enhance understanding and control of human diseases. In part 2 of the project, we will examine the requirement for linked recognition of covalently associated outer membrane proteins in generating immunity. *A. marginale* outer membrane proteins are covalently linked by extensive disulfide bonding. How this covalent bonding affects generation of immunity is unknown. The observation that Major Surface Protein (MSP) 1a-specific CD4+ T cells provide help for B-lymphocytes to secrete antibody to the covalently bound MSP1b suggests that the native association of outer membrane proteins can be critical in inducing protective responses. We hypothesize that this "linked recognition" of CD4+ T cell and B cell epitopes from different outer membrane proteins can be mimicked using chimeric multiple epitope constructs. At the conclusion of part 2, we expect to have determined if linked recognition between covalently bound outer membrane proteins, using MSP1a and MSP1b as a model complex immunogen, can be represented by a chimeric epitope construct.

Grant: 1R01AI053694-01
Program Director: KORPELA, JUKKA K.
Principal Investigator: COMSTOCK, LAURIE E
Title: Genetic Basis of Abscess Formation by *B. fragilis*
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 2003/09/30-2007/01/31

DESCRIPTION (provided by applicant): *Bacteroides fragilis* is the leading cause of anaerobic bacteremia and intraabdominal abscesses. The capsular polysaccharide complex of the prototype strain, 9343, confers the abscessogenic properties of the organism. The capsular polysaccharide complex of 9343 is comprised of at least eight distinct capsular polysaccharides (PSA1 - PSH1). For strain 9343, PSA1 is not only crucial for abscess formation, but is the only polysaccharide necessary for abscess formation by this organism. The region of the *B. fragilis* chromosome containing the PSA1 biosynthesis locus is heterogeneous, therefore, the PSA molecules synthesized by different *B. fragilis* strains are structurally distinct. It is not known whether the PSA molecules of other virulent strains also confer the abscessogenic potential of that organism. The zwitterionic nature of PSA1 of 9343, having both a positive and negative charge per repeating unit, is essential for its abscessogenic potential. Two genes of the PSA1 biosynthesis locus, *wcfR* and *wcfS*, are conserved in the PSA biosynthesis loci of all strains analyzed. Homology-based data suggest that the products of these genes are involved in the formation of the positively charged monosaccharide of PSA1, AATGal. Therefore, it is likely that all *B. fragilis* strains synthesize a PSA molecule with this same positively charged monosaccharide (AATGal). Based on the importance of AATGal to the virulence of the PSA1 of 9343, our overall hypothesis is that the PSA molecule confers the abscessogenic potential of each virulent strain. This application is divided into three aims that will address this hypothesis, first at the species level by mutating the PSA loci of various strains that produce structurally distinct PSA molecules and testing these mutants for their ability to induce abscesses. Next, the genes and their products that are predicted to be involved in the synthesis of AATGal will be analyzed biochemically and genetically. We now have the tools and an adequate scientific foundation to determine why the species *B. fragilis* as a whole has abscessogenic capabilities rather than only understanding this phenomenon for the prototype strain. The data gained from these aims may allow us to realize our goal of elucidating the genetic basis of abscess formation by this species.

Grant: 1R01AI053706-01
Program Director: HALL, ROBERT H.
Principal Investigator: SCHOOLNIK, GARY K MD
Title: Vibrio Cholerae Colonization of Chitin Surfaces
Institution: STANFORD UNIVERSITY STANFORD, CA
Project Period: 2003/09/30-2008/03/31

DESCRIPTION (provided by applicant): *Vibrio cholerae* O1, a cause of epidemic diarrheal disease, normally resides as an indigenous component of riverine, estuarine and marine ecosystems. In these habitats, it associates with the chitinous exoskeletons of zooplankton. The principal objective of this research project is to characterize the interaction of this human pathogen with a chitin surface. Chitin is an insoluble polymer of N-acetylglucosamine; through the secretion of chitinases, *V. cholerae* can use chitin as a sole source of carbon and nitrogen in nutrient-poor aquatic habitats. This project will explore the hypothesis that chitin utilization by *V. cholerae* is a four step process: chemotaxis toward and attachment to the chitin surface; horizontal dispersal of attached bacteria across the surface; vertical growth of the attached population as a biofilm community; and detachment from the biofilm surface and resumption of the planktonic mode-of-growth. The work proposed here will explore each of these hypothesized steps through the combined use of genomic, genetic and cell imaging methods. In collaboration with Professor Gill Geesey at Montana State University, reflected differential interference contrast (DIC) and epifluorescence microscopy will be used to image individual cells as they attach to and spread across a synthetic chitin membrane attached to a laminar flow cell experimental system. Scanning confocal laser microscopy (SCLM) will be employed at the Stanford Biofilm Center to capture the temporal and spatial features of biofilm development on the chitin surface. Microarray expression profiling will be used to identify genes, which comprise a hypothesized detachment regulon. Studies undertaken in collaboration with Professor Saul Roseman at Johns Hopkins University will examine the role of a newly identified chitin sensor histidine kinase protein in each of the four chitin utilization steps. Green fluorescent protein (GFP) promoter fusions will be used to disclose when and where individual genes are expressed on the chitin surface and mutants will be sought that are defective in the chitin utilization program. In the last Specific Aim of the proposed work, these results will be examined using experimental systems that more closely resemble conditions in natural aquatic ecosystems including their relevance for the colonization of swimming copepods. If successful, this project should generate new information about the persistence and control of infectious agents in aquatic reservoirs.

Grant: 1R01AI053728-01A1

Program Director: TAYLOR, CHRISTOPHER E.

Principal Investigator: GRIFFISS, JOHN M MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC

Title: A Meningococcal LOS Vaccine

Institution: NORTHERN CALIFORNIA INSTITUTE RES & SAN FRANCISCO, CA
EDUC

Project Period: 2003/06/15-2005/11/30

DESCRIPTION (provided by applicant): Meningococcal disease primarily affects infants and very young children. The group B capsule is not immunogenic, and outer membrane protein vaccines provide only short-lived protection, in older children, that is restricted to serotypes in the vaccine. Protection is mediated by bactericidal antibodies that are induced by asymptomatic colonization by organisms that share lipooligosaccharides (LOS). By adolescence most children have LOS IgG that are bactericidal for most meningococcal strains. These antibodies prevent meningococcal disease, regardless of protein serotype. LOS are immunogenic at birth, and LOS antibodies can be induced by vaccination. During disease, infants make bactericidal antibodies against their infecting strains. These antibodies bind to a conserved LOS structure that is expressed well by 126E (L1,8), other L1 strains, and by L3,7 and L4,6 strains. This structure has not been definitively identified. LOS antibodies induced during infancy can prevent the monoclonal antibody (mAb), D6A, from binding to 126E LOS, and we have used this mAb, which binds to meningococci of all groups and types, as a surrogate for the LOS structure. MAb D6A binds the deeply truncated LOS of deltagalE mutants that have only a conserved basal structure, but it is not clear that this truncated LOS is the optimal immunogen. We now propose to affinity purify human IgG that binds the conserved LOS structure by 1) passage of IVIG over deltagalE LOS, and 2) by sequential passage of IVIG over L1, L1,8, L3,7 and L4,6 LOS, each coupled to Sepharose. We will assess how well each IgG kills 34 consecutive and unique endemic meningococcal case strains, and whether they can opsonize these meningococci for PMN killing. We will compare the binding of this pauciclonal IgG (pclgG) to that of mAb D6A and use Mass Spectrometry combined with chemical and enzymatic degradations to confirm the LOS structure recognized by mAb D6A and pclgG. We will immunize transgenic mice that have human immunoglobulin loci (XenoMouse) with a deltagalE mutant to insure that this LOS structure is immunogenic for the human immune system, and characterize the functional activity of the induced IgG, as for the IVIG IgG. We will use enrichment of a coliphage display library with mAb D6A to identify a peptide mimic of the mAb D6A LOS antigen, use this peptide to immunize XenoMouse mice and functionally characterize the induced IgG. We also will try to identify a peptide mimic that binds the human pclgG. The resulting data should confirm the suitability of a conserved LOS structure as a vaccine for the prevention of endemic group B disease in infants and young children.

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Grant: 1R01AI053749-01
Program Director: KLEIN, DAVID L
Principal Investigator: HOLLINGSHEAD, SUSAN K
Title: Evolution of Virulence in *Streptococcus pneumoniae*
Institution: UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL
BIRMINGHAM
Project Period: 2002/12/01-2007/11/30

DESCRIPTION (provided by applicant): Transformation has been of key importance in the history of bacterial genetics in general and streptococcal genetics in particular and it is currently still the most important technique for genetic analysis of *Streptococcus pneumoniae*. Transformation is the major means of spread of antibiotic resistance in this species and the factors governing transformation in natural environments will be a key factor in the success of future vaccines and interventions. The goal of this grant is to address a simple question: What is the proportion of genomic information typically exchanged during transformation in the pneumococcus? Estimates based on experiments from the 60's and 70's are that this proportion is as high as 10% of the genome but those studies were based on a small number of marker genes and the results were not representative of the whole. The readout for genomic exchange in our experimental approach will be the proportion of strain-specific genes exchanged as measured by microarray. In Aim 1, some of the parameters that determine this proportion during in vitro transformation will be explored, including the length of donor DNA fragment and the concentration of donor DNA. In Aim 2, gene exchange from in vivo transformation will be followed retrospectively by looking at its extent following clonal divergence. For this purpose, the clonal complex (CC14) that includes serogroup 6 isolates in five world-wide multi drug-resistant lineages from 23 countries, will be studied. CC14 is associated with invasiveness in humans and mouse virulence vary across the lineage. Keeping in mind that virulence is multi factorial, this allows the detection of virulence-associated genes in the strain-specific gene sets. In Aim 3, in vivo transformation that has occurred during nasopharyngeal carriage in a mouse model will be examined. Isolates with recombinant genotypes will be detected by FACS sorting using markers to detect exchange in genes for two of the major virulence factors, capsule and *pspA* (pneumococcal surface protein A). By comparing the virulence of isolates within CC14 with their genes detected using a special microarray of strain-specific genes, we hope to also identify new virulence genes of pneumococci.

Grant: 1R01AI054442-01
Program Director: HALL, ROBERT H.
Principal Investigator: HUGHSON, FREDERICK M BS
Title: Structure-Function Analysis of AI-2 Quorum Sensing
Institution: PRINCETON UNIVERSITY PRINCETON, NJ
Project Period: 2003/03/15-2008/02/28

DESCRIPTION (provided by applicant): The long-term goal of this research is to explore the molecular mechanisms that bacteria use for cell-cell communication. Here we propose an integrative structural, chemical, and biological study of a recently identified quorum sensing circuit whose functioning depends on the production and detection of a small molecule signal called autoinducer-2 (AI-2). AI-2, unlike other known autoinducers, has the potential to mediate cell-cell communication among different bacterial species. To develop a molecular understanding of how AI-2 is detected, and how sensory information is transduced to control behavior on a community scale, we plan to carry out in-depth studies that combine synthetic organic chemistry, bacterial genetics, biochemistry, and X-ray crystallography. The proposed aims build on previous efforts by the investigators in which the crystal structure of an AI-2 sensor protein, LuxP, was determined in a complex with AI-2, revealing the bound ligand to be a furanosyl borate diester bearing no resemblance to previously characterized autoinducers. Specifically, the first aim is to use this structural information to design, synthesize, and functionally characterize quorum sensing agonists and antagonists. The second aim is to carry out genetic screens to identify dominant missense alleles of LuxP that are incapable of responding to AI-2. Gain-of-function LuxP mutants that signal in the absence of AI-2, or in the presence of AI-2 analogs, will also be identified. In the third specific aim, binding assays capable of measuring the affinity of AI-2 and AI-2 analogs for LuxP will be developed and used to evaluate the binding properties of wild-type and mutant LuxP proteins. In the fourth aim, X-ray structural studies of apo-LuxP, LuxP bound to quorum sensing agonists and antagonists, and LuxP homologs from other bacterial species will be carried out. It is anticipated that the closely coordinated multidisciplinary approach proposed here will lead to substantial new insights into bacterial quorum sensing via AI-2. These studies may also result in the discovery of compounds useful in the development of novel broad-spectrum antibacterial drugs that target quorum sensing.

Grant: 1R01AI054444-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: RAMAN, C S PHD
Title: Structural Biology of Prokaryotic NO Synthases
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX
HOUSTON
Project Period: 2003/03/01-2008/02/29

DESCRIPTION (provided by applicant): Nitric oxide (NO) has emerged as an important new gaseous messenger molecule that can mediate physiological and pathological responses in humans. NO regulates blood pressure in healthy human subjects and is also constantly synthesized by a healthy beating heart for its proper function. While controlled production of NO is essential for an individual's well being, an overproduction can lead to severe problems. In mammals NO is produced by a family of three enzymes collectively known as nitric oxide synthases (NOS). NOS is a bidomain protein made up of a heme-containing catalytic domain covalently fused to a reductase domain via a linker that binds calmodulin. Physiological actions of NO are mediated by its heme protein receptor, soluble guanylyl cyclase, which gets activated by NO and catalyzes the biosynthesis of second messenger, cGMP. NO can mediate its actions via both cGMP-dependent and -independent mechanisms. The latter includes the ability of NO to kill bacterial and viral pathogens. The toxicity of NO to bacteria has given rise to the assumption that NO signaling pathway is indigenous to eukaryotes. We have discovered that some gram-positive bacterial pathogens encode a NOS-like gene whose product bears strong sequence and structural resemblance to the catalytic heme domain of NOS found in mammals. The major long-term goal of this proposal is to understand the structural bases of catalysis and molecular recognition by bacterial NOS. We will particularly focus our efforts on NOS from *Bacillus anthracis*, the etiological agent of anthrax, and *Staphylococcus aureus*, an organism that exhibits remarkable resistance to antibiotics. A secondary long-term goal is to utilize bacterial NOS as a model system to gain molecular insights into the workings of mammalian NOS. A tertiary long-range goal is to understand the function of NOS in bacterial pathogens and to discover novel pathways in which it might participate. To achieve these goals, we plan to utilize a battery of X-ray crystallographic, biochemical, spectroscopic, molecular biological, and genetic methods. Knowledge gained from the proposed research will not only generate key information regarding how bacteria generate and deal with nitrogen oxides, but also provide us with critical leads for the development of novel strategies to combat biological warfare.

Grant: 1R01AI054474-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: LEWINSOHN, DEBORAH A MD
Title: Tuberculosis Immunity in Young Children
Institution: OREGON HEALTH & SCIENCE UNIVERSITY PORTLAND, OR
Project Period: 2003/05/15-2007/04/30

DESCRIPTION (provided by applicant): Tuberculosis (TB) is one of the most important causes of infectious morbidity and mortality worldwide. Young children are more likely to contract infection with and develop severe disease from the causative agent *Mycobacterium tuberculosis* (Mtb). These clinical observations likely reflect fundamental differences in the immune systems of young children and adults. Critical differences relevant to TB immunity include the propensity for infants and young children to develop TH2-type CD4+ T cells in response to immunogens, deficiencies in the development of TH1-type T cells in response to pathogens, and deficiencies in macrophage and dendritic cell (DC) function. We propose to systematically define, in young children (less than or equal to 10 years of age), these important differences relevant to the successful containment of Mtb infection. The specific aims are: 1) To determine if severity of disease following Mtb infection in young children is associated with TH2-type Mtb-specific immunity, and conversely, if absence of disease following Mtb infection is associated with TH 1-type Mtb-specific immunity. 2) To determine if immunologic immaturity is associated with the development of TH2-type Mtb-specific immunity following Mtb infection, and conversely if immunologic maturity is associated with the development of TH 1-type Mtb-specific immunity following Mtb infection. 3) To evaluate aspects of innate immunity relevant to Mtb infection by characterizing the phenotype and function of macrophages and DC from cord blood derived from healthy neonates in comparison to macrophage and DC function in healthy adults. These studies may contribute to a more complete understanding of TB immunity in young children, and hence 'facilitate the development of an improved TB vaccine for this vulnerable population.

Grant: 1R01AI054536-01
Program Director: HALL, ROBERT H.
Principal Investigator: DECATUR, AMY L PHD
Title: Host regulation of secreted, bacterial virulence factors
Institution: UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA
Project Period: 2003/09/30-2008/03/31

DESCRIPTION (provided by applicant): The long-term goal of this study is to understand how an intracellular pathogen interacts with its host to establish a protected niche in which the pathogen can replicate. For the bacterial pathogen *Listeria monocytogenes* establishing and maintaining its intracellular niche, the host cytosol, requires precise spatial regulation of an essential virulence factor, listeriolysin O (LLO). LLO is a secreted pore-forming protein that mediates bacterial escape from the host vacuole to the host cytosol. Despite being continuously secreted by the bacterium, LLO is active only in the host vacuole. We have identified a cis-acting sequence in the amino terminus of LLO that is necessary to restrict the activity of LLO to the vacuole. Mutants that lack this sequence fail to correctly compartmentalize LLO activity, permeabilize the host plasma membrane in addition to the vacuolar membrane, and consequently destroy their intracellular niche. Importantly, these mutants are avirulent in vivo. The above sequence is rich in the amino acids proline (P), glutamate (E), serine (S), and threonine (T) and thus resembles eukaryotic PEST sequences. PEST sequences can target eukaryotic proteins for phosphorylation and/or degradation. We have shown that a mutant LLO lacking the PEST region accumulates to higher intracellular levels than the wild type protein suggesting that this region may influence intracellular stability of LLO. In addition, we have shown that LLO is phosphorylated in the host cytosol and that mutants lacking potential phospho-acceptor sites located within the PEST sequence also permeabilize the host plasma membrane and have decreased virulence. The specific goal of this proposal is to understand the mechanism by which LLO's PEST sequence regulates the protein's activity in the host cytosol. In Aim 1, we will identify specific residues within LLO's PEST sequence that are important for its function and which may represent contact sites for interacting host molecules. In Aim 2, we will define the pathway of LLO degradation and determine whether the PEST sequence affects this pathway. Lastly, in Aim 3, we will define the role of intracellular phosphorylation of LLO. Controlling when and where a virulence factor acts is critical for an intracellular pathogen to orchestrate a productive infection and cause disease. By defining the mechanism by which LLO activity is compartmentalized within a host cell, we will learn more about how intracellular pathogens can take advantage of host cell machinery to regulate the activity of key virulence factors that function within the host cytosol.

Grant: 1R01AI054574-01
Program Director: ZOU, LANLING
Principal Investigator: PANG, YUAN-PING PHD
Title: Effective Countermeasures to Anthrax and Botulinum Toxin
Institution: MAYO CLINIC COLL OF MEDICINE, ROCHESTER, MN
ROCHESTER
Project Period: 2003/09/26-2005/12/31

DESCRIPTION (provided by applicant): One effective way to counter bioterrorism is to stockpile inhibitors that can selectively cripple enzymes pivotal to pathogens. Anthrax can be detoxified by selective inhibitors targeting anthrax's lethal factor (LF) that acts as a zinc protease. Botulinum toxin can be detoxified by selective inhibitors targeting its zinc endopeptidase. If such inhibitors were readily available, anthrax and botulinum would no longer be life threatening. The effectiveness of this approach rests on the fact that pathogens rely on enzymes. Furthermore, viral and bacterial enzymes have high substrate selectivity and can therefore be inhibited by selective inhibitors without interfering with other enzymes required for normal functions. To expeditiously develop selective enzyme inhibitors as countermeasures to biological weapons, we have recently developed i) an effective method for computationally simulating zinc proteases such as LF and botulinum neurotoxin serotype A (BoNTA), ii) an advanced computer program and a 1.1 teraflop supercomputer optimized for rapidly identifying enzyme inhibitors in silico, and iii) a database of 2.5 million unique chemical structures. In addition, we have computationally refined the 3D structures of LF and BoNTA. Our pilot study using these new technologies and the refined structures has already culminated in a small molecule that selectively inhibits BoNTA with an estimated IC (50) of 4 μ M and a weak small-molecule inhibitor of LF. Here we propose to develop potent and selective small-molecule inhibitors of LF and BoNTA as countermeasures to anthrax and botulinum toxin. We will develop the desired LF inhibitors by i) computationally identifying selective LF inhibitors from 2.5 million chemical structures, ii) synthesizing top 500 LF inhibitor candidates, iii) performing in vitro studies of 500 compounds, ex vivo studies of top 50 compounds, and in vivo studies of top 5 compounds, and iv) structural optimization of top 5 compounds with a combinatorial chemistry approach to obtain inhibitors with chemical and biological properties satisfying the criteria for clinic drugs. We will use the same strategy to develop BoNTA inhibitors as the one used for LF inhibitors. Successful completion of this proposal will lead to a timely treatment for intoxication caused by anthrax or botulinum toxin. It complements the efforts of other workers in developing methods for rapidly identifying anthrax and botulinum toxin.

Grant: 1R01AI054607-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: LEE, CHIA Y
Title: Global regulation by mgr in *S. aureus*
Institution: UNIVERSITY OF KANSAS MEDICAL CENTER KANSAS CITY, KS
Project Period: 2003/04/01-2008/03/31

DESCRIPTION (provided by applicant): *Staphylococcus aureus* is capable of causing a wide range of human diseases. It has become more problematic recently due to increasing resistance of the organism to antibiotic treatment. New methods of treatment are therefore urgently needed. The pathogenicity of this organism could be attributed to its ability to produce a large number of cell surface-associated and extracellular virulence factors. These virulence determinants are coordinately regulated by several unlinked global regulatory loci. Recently, we have identified a novel locus, mgr, which affects the production of several virulence factors in a pattern distinct from other systems reported to date. We have subsequently employed genetic and molecular methods to map and clone this global regulatory locus. Sequencing, gene-specific mutation and complementation confirmed that the mgr was a novel locus. Our data also suggest that mgrA is most likely the only gene in this locus that is required in the regulation. In this proposal, we propose to accomplish three specific aims: (i) to molecularly characterize the mgr locus by studying the transcriptional organization and analyzing the promoter region of the mgrA gene; (ii) to investigate the mechanism of target gene regulation by mgr and to identify additional target genes by micorarray technology; (iii) to study interaction with other global regulatory systems and control of mgrA gene expression. Various genetic, biochemical and molecular approaches will be employed to accomplish these aims. The successful completion of the studies outlined in this proposal will provide further insights into the mgr regulatory system. The new knowledge will undoubtedly contribute to our understanding of global regulatory mechanism in *S. aureus*, which will further provide a solid basis for the development of new treatments.

Grant: 1R01AI054624-01
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: DARVILLE, TONI MD
Title: Cell Death and Innate Immunity
Institution: UNIVERSITY OF ARKANSAS MED SCIS LTL LITTLE ROCK, AR
ROCK
Project Period: 2003/03/01-2008/02/29

DESCRIPTION (provided by applicant): Chlamydia species provoke serious infections of humans and animals worldwide, despite extensive work to better characterize the biology of the infection and develop vaccines. The biphasic developmental cycle of chlamydia allows for multiple sites of communication between the pathogen and the host cell; examples include the signals that block, and then later induce apoptosis. Apoptosis of chlamydia-infected cells triggered with external ligands is blocked through inhibition of cytochrome c release and caspase-3 activation, while apoptosis induced by the infection itself is independent of known caspases, and dependent on activation of the pro-apoptotic factor, BAX. Our data show that BAX translocates from the cytosol to mitochondria in infected cells, and inhibition or absence of BAX results in lower chlamydia-induced apoptosis and chlamydial propagation. This process has an intimate role in the host inflammatory response and tissue pathology as mice genetically deficient in BAX exhibit increased inflammation and tissue damage despite a lower level of infection. The overall hypothesis to be tested is that chlamydiae induce apoptosis as a quiet means of escape from the cell, but that infection-related cellular necrosis inevitably occurs, leading to the release of 'danger' signals and subsequent inflammation. The primary goal of these studies is to examine the entwined pathways of apoptosis and inflammation in vitro and in vivo as they relate to chlamydial disease pathogenesis. Our first two goals are to 1) determine mechanisms for, and roles of chlamydia induced apoptosis and chlamydia-induced protection from apoptosis in infection and disease, and 2) evaluate whether danger signals released from necrotic cells, such as ATP and adenosine, modulate infection and inflammation. Cellular recognition of pathogens initiates signals related to inflammation, as well as to cell survival. Thus, our last goal will be to 3) determine the contribution of Toll-like receptors (TLRs), cytosolic TLR-related proteins such as Nod1, and downstream signaling proteins in recognition of chlamydia and initiation of cellular responses in vitro and in vivo. Studies of chlamydia infection in cell lines and primary cell cultures from knockout mice will determine signaling events and proteins important in the innate immune response to chlamydia in vitro. The relevance of these data to disease pathogenesis will be determined with the use of knockout mice and an established mouse model of chlamydial genital tract disease.

Grant: 1R01AI054697-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: ORME, IAN
Title: Guinea pig model for TB vaccine evaluations
Institution: COLORADO STATE UNIVERSITY-FORT COLLINS, CO
COLLINS
Project Period: 2003/03/01-2008/02/28

DESCRIPTION (provided by applicant): Significant progress has been made over the past few years in identifying new vaccine candidates that could potentially be used for the prevention or immunotherapy of tuberculosis in human beings. The purpose of this proposed project is to take a select few of these vaccine candidates and perform longer term efficacy and safety evaluations to see which are the most robust. The animal model to be used will be the low dose aerosol challenge in the guinea pig, an animal which provides a stringent test given its susceptibility to infection and similarities in immunopathology to human disease. The central hypothesis to be tested will be that different vaccines, given data that their immunologic targets may differ, may behave differently in their ability to modulate, delay, or prevent the development of the disease process. To facilitate this analysis, we will use as a template our recent definition of four discrete phases of the granulomatous process in the lungs of infected guinea pigs. The proposed studies are lengthy, and unavoidably expensive, but should provide a basic framework upon which a standardized procedure for efficacy and safety testing of new tuberculosis vaccines can be based.

Grant: 1R01AI054796-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: HAN, JIAHUI PHD
Title: Molecular Mechanisms of Anthrax-Induced Macrophage Death
Institution: SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA
Project Period: 2003/02/15-2008/01/31

DESCRIPTION (provided by applicant): Exotoxins produced by anthrax bacilli are believed to be responsible for overt shock symptoms and death in infected animals. Cytolysis of macrophages caused by anthrax lethal toxin (LeTx) is a trigger of shock symptoms and death. LeTx can directly lyse macrophages of some mouse strains. However, the sensitivity of different murine macrophages to LeTx in vitro does not correlate with in vivo susceptibility of corresponding strains to *B. anthracis*. This suggests that there are factors other than LeTx also contributing to the cytolysis of macrophages. The death of LeTx-resistant macrophages needs to be studied because LeTx alone cannot kill human macrophages in vitro. The long-term goal of our study is to understand the molecular mechanisms that lead to the death of LeTx-resistant macrophages in anthrax infection. We have found that treatment of macrophages with bacterial components can make LeTx-resistant macrophages became sensitive to LeTx-induced cytolysis, suggesting that the death of LeTx-resistant macrophages requires two stimuli. We further determined that tumor necrosis factor- α (TNF) induced by bacterial components is at least one of the factors that can cooperate with LeTx in inducing macrophage death. In addition, mTor (mammalian target of rapamycin) signaling was found to be required for the death of LeTx-resistant macrophages. Although anthrax bacilli can escape phagocytosis by macrophages, they should activate macrophages to certain levels. We believed the autocrine effect of TNF plays a key role in LeTx-resistant macrophage death in vivo. In supporting this notion, it has been reported that administration of anti- TNF antibody improved survival of anthrax-infected C57BL/6 mice. This proposal will focus on the mechanisms of the cell death in LeTx-resistant macrophages. The death of LeTx-resistant macrophages will be addressed from the side of macrophage activation. The signaling pathways that cooperatively operate in causing LeTx-resistant macrophage death will be elucidated by biochemical and molecular biology approaches. The information obtained in this study will be very valuable in developing new strategies for the treatment of anthrax infection.

Grant: 1R01AI054929-01
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: CASTRIC, PETER A
Title: Pseudomonas aeruginosa 1244 Pilin Glycosylation
Institution: DUQUESNE UNIVERSITY PITTSBURGH, PA
Project Period: 2003/03/01-2008/02/28

DESCRIPTION (provided by applicant): Pseudomonas aeruginosa persists as a major cause of life-threatening infections for individuals with the following conditions: cystic fibrosis, burns, wounds, cancer (leukemias), those receiving immunosuppressive therapy, diabetics, as well as intravenous drug users. The pili of this bacterium are protein filaments that extend from the ends of the cell. These structures serve as adhesion factors, and so are important virulence factors. Work from my laboratory has shown that the pili of P. aeruginosa 1244 are glycosylated with the O-antigen repeating unit of this organism. This trisaccharide moiety originates in the lipopolysaccharide O-antigen biosynthetic pathway. The long-term objectives of this project are to determine the role of pilus glycosylation in P. aeruginosa pathogenesis and to ascertain the importance of the glycan in pilus vaccine design. The work described in this proposal aims to determine the glycan and the pilin substrates in the P. aeruginosa 1244 pilin glycosylation reaction. In addition, the subcellular location of PilO, the enzyme that catalyzes pilin glycosylation in P. aeruginosa will be established. The membrane topology of this enzyme will also be determined. Finally, the subcellular location of the P. aeruginosa 1244 pilin glycosylation reaction established.

Grant: 1R01AI054959-01
Program Director: VAN DE VERG, LILLIAN L.
Principal Investigator: VAZQUEZ-TORRES, ANDRES PHD
Title: Salmonella evasion of NADPH oxidase-dependent killing
Institution: UNIVERSITY OF COLORADO DENVER/HSC DENVER, CO
AURORA
Project Period: 2003/09/30-2007/12/31

DESCRIPTION (provided by applicant): The appearance of multidrug-resistant Salmonella isolates and the HIV epidemic have contributed to the resurgence of salmonellosis, an infection that annually afflicts more than 1 billion people worldwide. Multiple clinical and experimental lines of evidence point to the NADPH oxidase as a critical host defense mechanism in resistance to acute Salmonella infections. Salmonella, an enteric pathogen adapted to the intracellular environment of phagocytes, resides in remodeled phagosomes that selectively block contact with lysosomes and endocytic vesicles harboring the NADPH oxidase. A recently discovered locus at centisome 30 of the Salmonella chromosome encodes a type III secretory system known as Salmonella pathogenicity island 2 (SPI2) that disrupts maturation of the Salmonella phagosome. The primary goal of my laboratory is to understand the mechanisms by which this intracellular pathogen remodels its phagosome and evades the antimicrobial armamentarium of professional phagocytes. In the present proposal, we plan to test the hypothesis that SPI2 effectors decrease TNFRp55-stimulated ganglioside synthesis, thus blocking the migration of NADPH oxidase-harboring vesicles to the vicinity of the Salmonella phagosome. We specifically plan: 1) To identify SPI2 effector proteins that block trafficking of the NADPH oxidase. Attenuation of SPI2 mutants in macrophages and mice, coupled to techniques in molecular and cell biology, biochemistry and microscopy will be used to identify effector proteins that block NADPH oxidase trafficking. 2) To identify points in the TNFRp55-stimulated sphingomyelin pathway which are inhibited by SPI2 effector proteins. Lipid biochemistry, enzymology and bacterial genetics will be used to identify points in the sphingomyelin pathway inhibited by SPI2 effectors. And 3) To determine the kinetics of secretion and intracellular location of SPI2 effectors that inhibit the trafficking of the NADPH oxidase. Cell biology, immunology and microbial genetics will be used to study the early intracellular expression of SPI2 effectors and their distribution relative to the NADPH oxidase, TNFRp55 and the Salmonella phagosome. These studies will not only shed light on the cell biology of the NADPH oxidase but will also identify potential molecular targets common to intracellular pathogens such as Salmonella, Mycobacterium, and Legionella that are capable of thwarting the normal maturation of the nascent phagosome.

Grant: 1R01AI055042-01
Program Director: KORPELA, JUKKA K.
Principal Investigator: YAHR, TIMOTHY L PHD
Title: Regulation of *Pseudomonas aeruginosa* type III secretion
Institution: UNIVERSITY OF IOWA IOWA CITY, IA
Project Period: 2003/05/15-2008/04/30

DESCRIPTION (provided by applicant): *Pseudomonas aeruginosa* is an opportunistic pathogen of humans commonly isolated from patients with cystic fibrosis, nosocomial pneumonias, urinary tract infections, or severe burns. One of the many virulence determinants of *P. aeruginosa* is a type III secretion system. The type III system is required for pathogenesis in infection models of lung, cornea, and burned epithelia and functions to deliver the ExoS, ExoT, ExoU, and ExoY cytotoxins to the cytoplasm of eukaryotic host cells. Translocation of these toxins promotes evasion of host immune responses and dissemination of *P. aeruginosa* from sites of colonization. Expression of the type III system is highly regulated and induced through contact of *P. aeruginosa* with eukaryotic cells, growth in the presence of serum, or low Ca^{2+} concentrations. The mechanism of coupling these environmental cues to expression of the regulon are unclear. The long-term goal of these studies is to identify and characterize the mechanisms involved in regulation of the *P. aeruginosa* type III regulon. We have identified ExsD as a negative regulator of the type III regulon. ExsD is unique to *P. aeruginosa*, lacks a DNA-binding motif, and is coordinately regulated with the type III regulon. In an *exsD* mutant expression of the regulon is derepressed and overexpression of ExsD leads to repression of the regulon. We hypothesize that ExsD functions as a sensor of the type III translocase. Prior to contact of *P. aeruginosa* with eukaryotic host cells the type III secretion channel is closed and ExsD prevents expression of the type III system. Contact with host cells opens the type III translocase and triggers a mechanism for suppressing the negative regulatory activity of ExsD. Therefore, we hypothesize that the negative regulatory activity of ExsD is reflective of the secretion state of the bacterial cell. This would provide a simple and specific mechanism for induction of the type III system in response to contact of *P. aeruginosa* with eukaryotic cells. To test these hypotheses we propose the following specific aims; (1) Identify ExsD domains and residues important for negative regulation, (2) Identify and characterize ExsD interaction partners, and (3) Determine the regulatory role of ExsD in response to alternative environmental cues.

Grant: 1R01AI055052-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: GANTA, ROMAN R PHD
Title: Cellular Immunity Against Ehrlichia chaffeensis
Institution: KANSAS STATE UNIVERSITY MANHATTAN, KS
Project Period: 2003/09/30-2008/01/31

DESCRIPTION (provided by applicant): Human ehrlichiosis is caused by three agents including Ehrlichia chaffeensis, Anaplasma phagocytophila and by E. ewingii. These potentially fatal infections pose a serious threat to public health, particularly to immunocompromised and elderly people. However, little is known about the mechanisms of host resistance and reasons for the emergence of these diseases. This project proposes to characterize the host immune response necessary for clearance using mouse as the experimental host because the course of E. chaffeensis infection is similar between immunocompetent humans and mice and the model will provide data for human applications. Moreover, the extensive array of molecular, immunological and genetic tools available for mice will allow us to manipulate the system in ways that are not possible in other species. Specifically, three hypotheses will be tested: 1) TH1 type helper T-cells are required for clearance of E. chaffeensis; 2) Secreted cytokines from T-cells are needed for host resistance to E. chaffeensis infections; 3) Macrophage activation contributes to the clearance of E. chaffeensis. Several observations form the basis for this project: immunocompetent mice clear E. chaffeensis by 16 days and the clearance is associated with the expression of an E. chaffeensis-specific TH1 type IgG response, CTL response and granuloma formation; the infections are fatal in SCID mice lacking T- and B-cells; mice lacking functional MHCII genes establish long-term persistent infections after E. chaffeensis challenge; mice deficient for macrophage activation develop short-term persistent infections; and prior activation with IFN-gamma inhibits monocyte infections with E. chaffeensis. The importance of helper T-cells, cytotoxic T-cells, B-cell responses, cytokines, and macrophage activation will be evaluated using several mouse strains with different genetic backgrounds and by manipulating their immune systems to diminish or enhance particular immune components. Infections will be monitored using bacteriological, molecular, immunological, and pathological analyses to assess host immunity and infection status. The long-term goals of this project are to elucidate host immune mechanisms, pathogen evasion strategies and to ultimately use the information to devise effective intervention measures against E. chaffeensis and other closely related organisms.

Grant: 1R01AI055058-01
Program Director: HALL, ROBERT H.
Principal Investigator: CAMILLI, ANDREW BS
Title: Study of transmissible forms of *Vibrio cholerae*
Institution: TUFTS UNIVERSITY BOSTON BOSTON, MA
Project Period: 2003/05/15-2008/04/30

DESCRIPTION (provided by applicant): *Vibrio cholerae*, which lives in association with plankton in brackish, temperate waters the world over, is the causative agent of endemic and epidemic cholera. Hallmarks of the disease include prodigious watery diarrhea resulting from the action of secreted cholera toxin (CT), and infrequent but deadly explosive epidemics. The strong link between explosive epidemics and human crowding accompanied with untreated drinking water suggests a very efficient mode of fecal-oral transmission. We have discovered a heightened state of transmissibility of stool *V. cholerae* (referred to simply as "hyperinfectivity"), which persists even after shedding into water reservoirs. Knowledge of the molecular basis for this phenotype, and a general characterization of this transmissible form of *V. cholerae*, would contribute to the design of vaccines to prevent cholera at the initial stage of infection. Aim 1 of this proposal will use transcriptional profiling and proteomics to help define this transmissible form. Spotted DNA microarrays will be used to determine the transcriptome of stool *V. cholerae* incubated in pond water, and this will be compared to that of fresh stool *V. cholerae* to identify potential differences. The results will be validated by quantitatively assaying the steady state mRNA and protein levels from select genes. Microscopy and transcriptome data on stool *V. cholerae* predict a bacterial state of motility working in the absence of chemotactic signaling. This counterintuitive state is hypothesized to be responsible, at least in part, for the hyperinfective phenotype. In Aim 2 of this proposal, quantitative immunodetection using paralog-specific antisera will be used to test for reduced expression of all three CheW linker proteins and all three CheR methyltransferases in fresh and pond water-incubated stool *V. cholerae*, as is predicted by current transcriptome data. In addition, capillary tube chemotaxis assays will be performed directly on *V. cholerae* from these samples to substantiate this hypothesis. Aim 2 will also test a second hypothesis, that ToxR regulated factors, which are essential for pathogenesis, are not playing a role in the hyperinfectious state. Finally, Aim 3 will use mutation and infectivity analyses to determine if other metabolic, physiologic or phenotypic properties of the bacteria contribute to the hyperinfective phenotype or, alternatively, to colonization of an environmental planktonic host, *Anabaena variabilis*. These studies will establish a basis for understanding the hyperinfective phenotype, and the properties in general, that are exhibited by fresh and pond water-incubated stool *V. cholerae*. In turn, this knowledge will enhance our understanding of transmission of this and perhaps other water-borne pathogens, it will aid in the development of new cholera vaccines that target the antigens of 'incoming' vibrios, and it may suggest new approaches for the prevention of the dissemination of this lethal organism.

Grant: 1R01AI055298-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: SLAYDEN, RICHARD A PHD
Title: Regulation of Cellular division in M. tuberculosis
Institution: COLORADO STATE UNIVERSITY-FORT FORT COLLINS, CO
COLLINS
Project Period: 2003/03/01-2007/02/28

DESCRIPTION (provided by applicant): This Proposal is in response to PA-01-113, "Therapeutics Research on AIDS-Associated Opportunistic Infections and Malignancies" and specifically addresses the study of Mycobacterium tuberculosis (MTB). The thrust of this proposal is the development of novel drug targets to counteract multiple drug resistant organisms. The long-term goal of this research program is to develop novel classes of chemotherapeutics that target the regulation, and coordination of chromosomal segregation and cellular division in MTB. Toward this objective we have identified in the MTB genome, gene products that are homologous to proteins associated with these processes in other prokaryotes. Moreover, our preliminary results provide strong evidence that some of these gene products (FtsZ and FtsI homologues) actively participate in the cellular division of MTB. However, a more extensive analysis of these gene products and global assessment of the potential regulatory networks involved in the division of MTB cells are required. Similar to work with *Caulobacter crescentus* we hypothesize that DNA microarray analysis with synchronized cultures of MTB will allow us to develop a detailed pattern of gene expression profiles across the entire cell division cycle of this bacterium. Additionally, the use of known inhibitors of early (FtsZ activity) and late (FtsI activity) events of cell division along with global gene expression studies will further elucidate the regulatory networks that are activated during different stages of cell division. A final analysis of putative regulatory genes already identified and new ones elucidated through gene expression profiling will enable us to develop a detailed picture of the regulatory networks and temporal gene expression responsible for MTB cellular division. Such studies will ensure that future resources are well directed at appropriate chemotherapeutic targets and developing suitable drug discovery strategies. Thus, the studies proposed in this application are designed to examine the replication dynamics of MTB, specifically focusing on cell cycle-regulated genes that are involved in cell division.

Grant: 1R01AI055377-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: SALGAME, PADMINI PHD
Title: Induction of Th1 Immunity in Tuberculosis
Institution: TEMPLE UNIVERSITY PHILADELPHIA, PA
Project Period: 2003/02/01-2004/01/31

DESCRIPTION (provided by applicant): Prevention of infectious diseases through vaccination remains a priority goal for scientists and public health officials. The high risk of developing multidrug-resistant Mycobacterium tuberculosis is a serious limitation to drug therapy, and consequently development of efficacious vaccines is critical for the successful eradication of tuberculosis. Vaccination against tuberculosis would rely on the immune system's ability to generate an appropriate protective response that can then be successfully garnered when the host is challenged with M. tuberculosis. Although we know that Th1 immunity is critical for protection against tuberculosis, yet the cells and mechanisms that are involved in generating a protective Th1 immunity are far from completely understood. In this proposal we combine two complementary approaches, a hypothesis-driven and low-through put approach and a global microarray analysis to ask mechanistic questions regarding how protective Th1 immunity to M. tuberculosis is generated. Therefore the central goal of this proposal is to examine the mechanism for the distinct cytokine response from dendritic cells and macrophages following M. tuberculosis infection, and study its impact on the development of M. tuberculosis-reactive CD4 + Th1 cells in vivo: from naive to effector. Overall we expect to obtain from these studies an integrated appreciation of the dynamics of induction of Th1 immunity in tuberculosis. Successful completion of these studies will provide innovative strategies for vaccine development and also provide new modalities of modulating the immune response to shorten chemotherapy and/or overcome drug resistance. The specific aims that will address the goals of the proposal are: Aim 1. Corroborate the theory that signaling from TLRs is sufficient for IL-12 production from dendritic cells, and that macrophages require an additional signal to synergize with TLR signaling for IL-12 production. Aim 2. Examine the molecular basis for differential IL-12 regulation in M.tuberculosis stimulated dendritic cells and macrophages. Aim 3. Test the hypothesis that dendritic cells are critical for Th1 cell priming, and that macrophages are elemental for initiating the granulomatous response. Aim 4. Compare the reprogramming of the dendritic cell and macrophage transcriptome in response to M.tuberculosis alone and in response to M.tuberculosis and interferon-gamma.

Grant: 1R01AI055496-01
Program Director: KORPELA, JUKKA K.
Principal Investigator: MOBASHERY, SHAHRIAR PHD
Title: RIBOSOMAL FUNCTION & ANTIBIOTIC DESIGN
Institution: WAYNE STATE UNIVERSITY DETROIT, MI
Project Period: 2003/07/01-2003/07/31

DESCRIPTION (provided by applicant): The recent availability of several x-ray crystal structures for the ribosome have created considerable excitement in the field. These structures will be instrumental in devising experiments to elucidate the details of ribosomal synthesis of proteins. Furthermore, the bacterial ribosome is the target of many classes of antibiotics, each of which interferes with an aspect of the biochemistry of protein synthesis. Studies of ribosome structures will lead the way in developing novel classes of antibiotics in the near future. The long-term goal of this multidisciplinary collaborative project is to utilize the structural information from known x-ray structures to study ribosome function in greater detail and to develop novel inhibitors of an essential and universal biosynthetic process. Four specific aims are proposed. 1) A combination of molecular dynamics simulations and experiments will be used to explore the dynamic nature of the decoding process during peptide-bond formation and to understand the role of antibiotic binding in this process. 2) Two molecules that have been designed to explore the motion of the rRNA A site during the decoding process will be synthesized and studied for rRNA binding. These compounds are potential inhibitors of A site function, hence possible antibacterials. 3) Two iron complexes of aminoglycoside-EDTA complexes will be synthesized and tested for their ability to bind the ribosome and fragment the rRNA backbone at sites located within the antibiotic-binding site(s). These experiments will identify the locations in which these antibiotics bind, as well as provide information about dynamics through examination of cleavage patterns. 4) Specific aim 4 expands on earlier success in the design of new antibiotics. A series of molecules that will interfere with protein synthesis by binding to the ribosomal A site will be generated. A host of specific analyses (assessment of antibacterial properties with living bacteria, translation assays, bacterial membrane permeability assay, assays for DNA and RNA function in bacteria, cytotoxicity assays, assays with A-site mutated 30S ribosomes and bacteria that harbor them, and x-ray analyses of the 30S ribosome and the A-site model complexed with the novel antibiotics) have been envisioned for the study of the antibacterial properties of these molecules. This collective effort will shed light both on the various molecular events within the ribosomal and on how to inhibit some of these processes.

Grant: 1R01AI055556-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: WARD, ELIZABETH SALLY PHD
Title: Antibody engineering: targeting Bacillus anthracis
Institution: UNIVERSITY OF TEXAS SW MED DALLAS, TX
CTR/DALLAS
Project Period: 2003/09/15-2007/12/31

DESCRIPTION (provided by applicant): Bacillus anthracis poses an enormous bioterrorism threat. Although antibiotics can be effective in treating anthrax infection, early diagnosis is essential. In addition, antibiotic resistant strains have been developed. There is also concern about the efficacy and safety of existing vaccines, and safety issues are exacerbated for the vaccination of neonates. Passive immunotherapy with antibodies would provide an attractive, alternative route for protection that would be effective against antibiotic resistant strains. However, suitable (humanized) antibodies are currently not available. The use of passive antibodies in prophylaxis also necessitates frequent doses due to the limited serum half-life of existing antibodies. We will attempt to overcome these shortcomings. First, we plan to use current methods of antibody engineering to generate antibodies that target both spore and toxin components of B. anthracis. Second, we will use technology that has been developed in our laboratory to increase the serum half-life of gamma globulins (IgGs). We have shown that it is possible to increase the serum persistence by engineering the site of an antibody that interacts with the Fc receptor, FcRn. This Fc receptor also regulates the transfer of IgG across the materno-fetal barrier. It is therefore likely that antibodies with increased serum half-lives will be transferred more efficiently to the fetus via increased binding to FcRn. Our specific aims are: 1) to generate effective anti-protective antigen and anti-spore coat protein antibodies; 2) to increase the serum half-lives of the antibodies; 3) to analyze the transfer of protective antibodies across human and murine maternal-fetal barriers; 4) to humanize the most promising antibodies for use in further studies. The proposed analyses should provide effective reagents for the prophylaxis and treatment of anthrax. They should also give new insight into understanding the role of antibodies in limiting infection, and this has broader relevance to other pathogens.

Grant: 1R01AI055578-01
Program Director: TAYLOR, KATHERINE A.
Principal Investigator: SHONE, CLIFFORD C PHD
Title: Sensitive Assays for the Botulinum Neurotoxins
Institution: CENTRE FOR APPLIED SALISBURY,
MICROBIOLOGY/RESEARCH
Project Period: 2003/09/01-2005/12/31

DESCRIPTION (provided by applicant): The botulinum neurotoxins are potent neuromuscular agents which pose a threat to human health both in natural outbreak occurrences and through deliberate release by bioterrorists. The currently accepted method of assay for the botulinum toxins, the mouse bioassay, is non-specific and too slow to provide a reliable diagnostic tool for use in an emergency response capacity. Rapid, sensitive and reliable in vitro assays are urgently required. Through a better understanding of the enzymology of the botulinum neurotoxins, the proposed study aims to develop sensitive in vitro assays for all seven toxin serotypes which can replace the mouse test. Target characteristics for the assay are:-- assay sensitivities equivalent to the mouse bioassay - applicable to a wide range of media including food extracts and serum- very low incidence of either false positive or false negative results - assay result in less than 6 hours. To achieve the required sensitivity (as low as 10 picograms/ml for botulinum neurotoxin serotypes A and B), assays will be developed which exploit a biological activity essential to the action of each neurotoxin. In these assay systems, the unique endopeptidase activity contained within the light chain of each botulinum neurotoxin will be used to amplify the assay to the desired sensitivity. This approach offers a number of advantages. Firstly, since the amplification system relies on the toxin itself, the requirement for specialized assay reagents is minimized. Secondly, since the assay signal depends upon a unique endopeptidase activity, the incidence of false-positive results will be very low. Lastly, since the assay is based on an essential biological activity within the neurotoxin, it closely resembles the mouse bioassay in that denatured toxin will not be detected. A prime goal of the research is to study further the enzymatic properties of the botulinum toxins and through this research develop endopeptidase-based, in vitro assays for each of the toxin serotypes and combine these into a simple, reliable test protocol.

Grant: 1R01AI055588-01
Program Director: KORPELA, JUKKA K.
Principal Investigator: ZHOU, PEI PHD
Title: Structure and Mechanism of LpxC in Lipid A Biosynthesis
Institution: DUKE UNIVERSITY DURHAM, NC
Project Period: 2003/06/15-2006/11/30

DESCRIPTION (provided by applicant): Lipid A, the hydrophobic anchor of lipopolysaccharide (LPS), is a glucosamine-based phospholipid that constitutes the outer monolayer of the outer membrane of most Gram-negative bacteria. Also known as endotoxin, lipid A is the active component of LPS that stimulates the immune system and causes lifethreatening Gram-negative septic shock, a severe condition characterized by disseminated intra-vascular coagulation and multiple organ failure. Lipid A biosynthesis is an essential pathway that is conserved in virtually all Gram-negative organisms. The committed step of lipid A biosynthesis is catalyzed by UDP-3-O- (acyl)-N-acetylglucosamine deacetylase (LpxC). LpxC belongs to a novel family of zinc-dependent metalloamidases and shares no sequence homology with any known mammalian proteins. Hence, it is an excellent target for the design of novel antibiotics. Indeed, inhibition of LpxC causes rapid bacterial death and cures mice infected with a lethal intraperitoneal dose of *Escherichia coli* (E. coli). However, potent inhibitors against the LpxC from *E. coli* are relatively inactive against divergent LpxCs from other Gram-negative bacteria, particularly, those from *Aquifex aeolicus* and *Pseudomonas aeruginosa*. Although LpxCs have been the subject of extensive biochemical studies and pharmacological screenings, the unusual inhibitor specificity and the lack of structural information on LpxCs and their complexes, either with substrates or inhibitors, hinder further mechanistic studies on LpxCs and the optimization of their inhibitors. The overall goal of this proposal is to reveal the largely unknown molecular mechanism underlying LpxC catalysis in lipid A biosynthesis and to provide a structural basis to rationalize the specificity of LpxCs from different Gram-negative species. These studies should also facilitate the development of novel antibiotics targeting LpxC. In the proposed work, the specific aims are: 1) determining the solution structure of the LpxC from *Aquifex aeolicus* (AaLpxC); 2) determining the solution structure of the AaLpxC/TU-514 inhibitor complex and characterizing the interaction between AaLpxC and its substrate; 3) determining the solution structure of the LpxC from *E. coli* (EcLpxC); 4) characterizing the interactions between EcLpxC and various inhibitors using structural and biochemical approaches.

Grant: 1R01AI055605-01
Program Director: RUBIN, FRAN A.
Principal Investigator: SCOTT, JUNE R
PHD
MICROBIOLOGY:BACTERIOLOGY
Title: Protein Export and Surface Anchoring in Gram+ Bacteria
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 2003/09/30-2008/01/31

DESCRIPTION (provided by applicant): Among the low G+C Gram+ bacteria are many important human pathogens, including some classified as potential bioterrorism agents (i.e. *Bacillus anthracis*, category A and *Listeria monocytogenes*, category B). Many of these organisms have proteins on their surface that lack typical Sec-dependent N-terminal secretion signals and thus use unknown mechanisms for export through the cell membrane (cm). Some of these proteins are apparently involved in pathogenesis since they are effective for passive immunization of mice. Aims 1 and 2 of this work are directed at identification and characterization of new Sec-independent protein secretion systems in these organisms. Once secreted through the cm in Gram+ organisms, proteins are either released into the extracellular milieu or anchored to the cell surface. The general mechanism for anchoring proteins to the cell wall (cw) in Gram+ bacteria requires a transpeptidase, called sortase, that recognizes and cleaves a short amino acid motif preceding a C-terminal hydrophobic region and charged tail. We recently characterized two sortases in the group A streptococcus (GAS) that anchor distinct subsets of proteins with the LPXTG motif. At a similar location in the genome of other GAS strains, we have identified genes encoding additional proteins with homology to sortase. In Aim 3 we will characterize the function in the GAS of these proposed new sortases and test their ability to anchor their predicted substrate proteins, which we find encoded nearby. We will also characterize a predicted protein with homology to signal peptidase I (encoded in the same region) that we propose will be needed for secretion of these proteins. The greater understanding of secretion and cell wall anchoring in Gram+ bacteria that results from this work should provide new targets for broad spectrum antibacterial therapy and potential new vaccine vectors. In addition, it should provide commercially useful new methods for large-scale production of proteins.

Grant: 1R01AI055612-01
Program Director: SCHMITT, CLARE K.
Principal Investigator: STINTZI, ALAIN C PHD
Title: Campylobacter colonization and virulence determinants
Institution: OKLAHOMA STATE UNIVERSITY STILLWATER, OK
STILLWATER
Project Period: 2003/09/30-2008/01/31

DESCRIPTION (provided by applicant): Campylobacter spp, generally food-borne, are the leading cause of gastroenteritis worldwide, surpassing the number of cases of Salmonella and Shigella combined. Campylobacter spp. are also associated with the development of Guillain-Barre syndrome, which is the most common cause of acute neuromuscular paralysis. In addition, since Campylobacter could be easily acquired and spread through our food supply, it constitutes a potential bioterrorism threat. Currently, no vaccine is available against Campylobacter infection, and despite an intensive research effort to understand Campylobacter pathophysiology, conclusions on the exact mechanism of infection are extremely difficult to draw. *C. jejuni* is adapted to survive both in the environment (mainly water and milk) and in its host organisms (mammals and birds). Upon entrance into the human host, Campylobacter must survive in the intestinal tract, either as a free bacterium in the mucus layer or intracellularly in gut epithelial cells. To colonize the intestinal tract, *C. jejuni* must successfully transit through the gastric acid barrier of the stomach to the more alkaline environment of the intestine. While up to 500 commensal species as well as other food-borne pathogens must similarly surmount these host barriers and adapt to the gut environment, very little is known about this process. This proposal focuses on the characterization of Campylobacter jejuni colonization and virulence factors. This proposal is based on the following hypothesis: there are numerous genes expressed in vivo that are influenced by environmental factors, and several of these genes are required for gut colonization and ultimately disease development. *C. jejuni* colonization and virulence determinants will be identified by in vitro and in vivo survival analysis of insertional mutants using DNA microarray. First, a functional genomic tool will be developed to identify conditionally essential genes in *C. jejuni*, and validated to examine the mechanism of *C. jejuni* survival to acid stress. Second, this functional genomic tool will be used to characterize the interactions of *C. jejuni* with the host gastrointestinal tract using the newborn piglet as an animal model of human infection. Finally, the role of the colonization and virulence determinants in disease and Campylobacter physiology will be assessed using a battery of in vitro biological assays. The identification of these Campylobacter determinants could significantly contribute to the development of more effective methods to diagnose, manage and ultimately prevent Campylobacter infections.

Grant: 1R01AI055614-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: BROWN, ERIC J MD CLINICAL MEDICAL SCIENCES, OTHER
Title: Mycobacteria Invasion and Persistence
Institution: UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA
Project Period: 2003/07/01-2007/12/31

DESCRIPTION (provided by applicant): Mycobacteria infections remain a major problem in human health. Mycobacterium tuberculosis infects approximately 1/3 of the world's population; Mycobacterium leprae (ML) is an endemic infection in many parts of the world. M. marinum (Mm) is a natural pathogen of fish and can cause chronic disease in frogs with many features of tuberculosis. Because of this, its rapid growth in vitro, its suitability for forward genetics, its minimal pathogenicity for laboratory workers, and its ability to grow in mammalian macrophages in vitro, Mm can be a facile model to discover genes involved in pathogenesis of Mycobacteria infections. We have developed a transposon mutagenesis plasmid for use in Mm that has proven to provide an excellent library of random mutations. From that random insertion library, we have screened >1000 mutants for ability to grow in macrophages and have characterized >25 mutants that fail to grow normally. For each mutant, we have sequenced the transposon insertion site, and, using this information, we have discovered several genes required for intracellular growth of Mm. We propose to focus on a more detailed characterization of two most interesting mutants to understand the roles of the targeted genes in host cell invasion by, and intracellular growth of, Mycobacteria. In each case, we have shown that the Mtb homologue of the targeted Mm genes will complement the phenotypic defects discovered in the mutant. This allows a rapid and thorough investigation of the roles of these Mtb genes in a model that is capable of intracellular growth and ultimately of extension to in vivo models of infection. The purpose of the current proposal is to use these mutants to develop a better understanding of Mycobacteria entry into and survival in macrophages, and of macrophage response to Mycobacteria infection, critical events in the pathogenesis of disease. Our specific aims are: 1. Characterize the mip locus, required by Mm for host cell invasion and intracellular survival in macrophages. 2. Determine how the GDP-mannose synthesis operon regulates macrophage activation and intracellular survival. 3. Carry out a genetic screen for Mm genes specifically required for intracellular growth in macrophages.

Grant: 1R01AI055715-01
Program Director: SCHMITT, CLARE K.
Principal Investigator: THOMPSON, STUART A BS
Title: Campylobacter jejuni proteins induced at 37C
Institution: MEDICAL COLLEGE OF GEORGIA (MCG) AUGUSTA, GA
Project Period: 2003/09/30-2008/01/31

DESCRIPTION (provided by applicant): Campylobacter jejuni is the leading cause of severe bacterial gastroenteritis in the U.S., and has been classified as a food-borne Category B Bioterrorism agent by the NIH. In addition to the tremendous burden of disease due to severe gastroenteritis (>2.4 million cases/yr, in the U.S.), C. jejuni infection is highly associated with the development of Guillain-Barre syndrome, an acute motor paralysis that may result from autoimmune antibodies against C. jejuni antigens. Poultry flocks are ubiquitously and asymptotically colonized with C. jejuni, and the most probable route of transmission of C. jejuni to humans is probably via consumption of contaminated poultry meat. In its natural habitats, C. jejuni is able to thrive at two different temperatures, 42C (the core temperature of chickens) and 37C (in humans). Consequently, there is likely to be temperature regulation of C. jejuni proteins to facilitate the optimal expression of the subset of proteins appropriate for its current environment (i.e., poultry or humans). Using complementary microarray and proteomics approaches, we have evidence that such temperature regulation occurs. Furthermore, C. jejuni temperature regulation may increase the expression at 37C of proteins that may be important in the course of human disease, and appear to define global regulatory networks that allow the simultaneous regulation of many C. jejuni proteins. We now propose further study of temperature regulation in C. jejuni, focusing on those proteins that are induced at 37C and which may be required for C. jejuni to cause disease in humans. We will achieve these goals using the following 3 specific aims: Specific Aim 1. Using proteomics and microarray, identify and localize C. jejuni proteins that are induced at 37C, and examine interstrain variability in 37C-induced proteins. Specific Aim 2. Characterize the functions and regulation of C. jejuni proteins that are induced at 37C. Specific Aim 3. Elucidate the roles of specific 37C-induced proteins in human epithelial cell binding and invasion in vitro, and in colonization in a mouse model.

Grant: 1R01AI055844-01
Program Director: ZOU, LANLING
Principal Investigator: CRYSTAL, RONALD G
Title: Anti-Y. pestis Vaccination and Passive Protection
Institution: WEILL MEDICAL COLLEGE OF CORNELL NEW YORK, NY
UNIV
Project Period: 2003/07/01-2007/12/31

DESCRIPTION (provided by applicant): Plague is caused by infection with the bacterium *Yersinia pestis*, a category A bioterrorism agent. Any strategy to protect against *Y. pestis* must recognize that the pneumonic form of the disease develops within days, and thus anti-*Y. pestis* protection has to be functional within days following an attack. This proposal presents a strategy for both rapid and long lasting protection against *Y. pestis* based on our experience in using adenovirus (Ad) gene transfer vectors to develop anti-bacterial vaccines, and the ability of Ad vectors to code for single chain antibodies against specific antigens. The underlying concept is that Ad-based gene transfer vectors can be used to evoke systemic, robust acquired immunity, as well as rapid passive immunity against *Y. pestis* antigens, and that both forms of protection can be achieved with a single administration of a single vector. The proposal uses an in vivo gene transfer-based strategy with a single Ad vector to simultaneously evoke rapid humoral immunity against *Y. pestis* (via an anti-*Y. pestis* single chain antibody coded by the vector), while also functioning as a vaccine to evoke endogenous host responses against *Y. pestis* antigens (via *Y. pestis* antigens coded by the vector). The 3 specific aims outline studies to achieve these goals by developing the vaccine and single chain antibody strategies independently, and then combined. Aim 1. To evaluate the hypothesis that a vaccine based on a replication deficient Ad vector encoding the *Y. pestis* V and F1 antigens will evoke robust systemic humoral immunity against these antigens and protect against challenge with *Y. pestis*. Aim 2. To assess the hypothesis that an Ad vector encoding a single chain antibody against an epitope of *Y. pestis* V or F1 antigen will provide robust, rapid humoral immunity against these antigens and protect against challenge with *Y. pestis*. Aim 3. To examine the hypothesis that a combined passive and active anti-*Y. pestis* protection can be achieved with a single administration of a single Ad vector expressing anti-*Y. pestis* single chain antibody and *Y. pestis* antigens.

Grant: 1R01AI055860-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: HOCH, JAMES A PHD
MICROBIOLOGY:MICROBIO
OGY-UNSPEC
Title: Signal Transduction Networks in Bacillus anthracis
Institution: SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA
Project Period: 2003/09/01-2007/02/28

DESCRIPTION (provided by applicant): Anthrax is a potentially fatal human disease caused by the Gram-positive spore-forming bacterium *Bacillus anthracis*. Virulence factors including the toxin crucial for *B. anthracis* pathogenesis are under the control of the phosphorelay signal transduction pathway that regulates post-exponential gene expression and sporulation through phosphorylation of the Spo0A response regulator transcription factor. Toxin proteins are exported to the outside of the bacterial cell where they exert their lethal effect. The research in this proposal is toward understanding anthrax toxin gene regulation and toxin secretion in order to identify new therapeutic targets for intervention and persistence. Proposed is the development of new and facile methods for gene inactivation, allele exchange and regulated gene expression in *B. anthracis* in order to dissect and identify those genes regulating toxin synthesis. Studies are envisioned to evaluate these genes in a pathogenesis model system leading to a more complete picture of the regulation of virulence factor synthesis and pathogenesis. The sensor histidine kinases that regulate the flow of phosphoryl groups through the phosphorelay to ultimately regulate toxin synthesis will be targets of investigation. Experiments are proposed to identify the signals recognized by *B. anthracis* that induce the phosphorelay. Genetic and biochemical approaches to the understanding of the mechanism by which toxin protein precursors are processed and secreted through the cytoplasmic membrane are proposed. The roles of the multiple signal peptidases in the secretion of toxin and other secreted proteins will be determined. Extensive physical characterization of the AbrB transition state regulator that represses toxin production will be undertaken to decipher how it is able to recognize seemingly random but specific DNA sequences in promoters. A combined in vivo and structural approach is proposed to pinpoint sites for intervention in the function of regulatory proteins leading to the synthesis of active anti-infective agents. Initial proof of concept studies will focus on the Spo0A response regulator for which extensive structural and biochemical information exists. This is a collaborative effort between investigators with expertise in genetics, biochemistry and structure of *Bacillus* regulatory proteins.

Grant: 1R01AI055894-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: IZARD, TINA PHD
Title: Human and Tuberculin CoA Biosynthetic Enzyme Structures
Institution: ST. JUDE CHILDREN'S RESEARCH HOSPITAL MEMPHIS, TN
Project Period: 2003/09/01-2008/02/29

DESCRIPTION (provided by applicant): Coenzyme A (CoA) is the major acyl group carrier for all organisms and is essential for cell survival. In bacteria, the last two reactions of CoA biosynthesis are mediated by the enzymes phosphopantetheine adenylyltransferase (PPAT), which generates dephospho-CoA (dPCoA), and dephospho-CoA kinase (DPCK), which yields CoA. In contrast, in higher eukaryotes a bifunctional enzyme, CoA synthase, catalyzes both of these final reactions. The substrate of PPAT is rate-limiting and the enzyme is feedback regulated by CoA, indicating that PPAT is an excellent target for developing novel antibiotics. We have solved the crystal structures of PPAT from *Escherichia coli*, which exists as a hexamer, and of PPAT bound to its substrates and to its product dPCoA. These studies identified the residues involved in substrate/product binding and revealed an in-line displacement catalytic mechanism. Finally, our Preliminary Studies of the PPAT:CoA crystal structure revealed an unusual mechanism of feedback inhibition of PPAT by CoA. The structure of the monomeric DPCK enzyme (from *Haemophilus influenzae*) has recently been solved and suggests an induced-fit reaction mechanism typical of kinases. Collectively, these structures form the foundation for the design of PPAT- and DPCK-specific inhibitors, an important issue in the context of treating drug-resistant bacteria, in particular tuberculosis. Like other bacteria, *Mycobacterium tuberculosis* requires PPAT and DPCK, and although the catalytic mechanisms of tuberculin PPAT and DPCK are likely identical to their homologs in other bacteria, there are significant differences predicted for the structures of these enzymes, especially DPCK. Recombinant *M. tuberculosis* PPAT and DPCK proteins have been produced and we have crystallized tuberculin PPAT. Experiments in Specific Aims #1 and #2 will determine the native and substrate-bound crystal structures of *M. tuberculosis* PPAT and DPCK. In addition, the structures of tuberculin PPAT bound to its product dPCoA, and to its inhibitor CoA will be solved. The design of PPAT- and DPCK-specific inhibitors requires that they not inhibit human CoA synthase. The monomeric nature, predicted structure, and coupled catalytic functions of CoA synthase indicate that regulation of this enzyme is unique, and a novel N-terminal domain may play a regulatory role. Experiments in Aim #3 will determine the native and substrate-bound crystal structures of human CoA synthase. The proposed crystallographic studies are essential for the development of novel inhibitors that selectively target *M. tuberculosis* PPAT and DPCK.

Grant: 1R01AI055913-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: WOOD, DAVID O PHD MICROBIOLOGY, OTHER
Title: Global Analysis of the Rickettsia prowazekii Proteome
Institution: UNIVERSITY OF SOUTH ALABAMA MOBILE, AL
Project Period: 2003/07/01-2007/12/31

DESCRIPTION (provided by applicant): Members of the genus Rickettsia are the etiologic agents of epidemic and endemic typhus and rocky mountain and other spotted fevers. These diseases pose significant health threats worldwide. Rickettsia prowazekii, the etiologic agent of epidemic typhus, is an obligate intracellular parasitic bacterium that can grow only within the cytoplasm of a eukaryotic host cell. R. prowazekii is able to exploit this intracellular niche in animals as diverse as arthropods and humans, allowing it to be vectored by the human body louse. This proposal is focused on the characterization of the protein expression patterns of R. prowazekii. Important targets for a proteome approach are the bacterial pathogens of humans, organisms that exhibit unique biology's, and unfortunately, in the current human environment, potential bioterrorist agents. R. prowazekii fits all three of these criteria and in addition, due to its small proteome, can serve as a model for complete proteome analysis. The specific focus of this proposal is on the development and application of proteomic techniques to address questions about the pathogen R. prowazekii. In Specific Aim 1 our goal is to develop and apply high-throughput, global liquid chromatography-mass spectrometry technology to obtain the complete proteome of the extensively studied R. prowazekii Madrid E strain propagated under standard conditions. We will apply this technology in Specific Aim 2 to identify global patterns of rickettsial protein expression. Rickettsial proteins expressed under different conditions that reflect the unique biology of this pathogen will be examined. These will include strain differences, host cell differences, temperature variation, and changes resulting from rickettsia growth. In Specific Aim 3 we will address a critical extension of host cell variation by examining the proteome of R. prowazekii growing in an arthropod vector. This interface is a critical component of rickettsial survival. Identification of differences in rickettsial protein expression will increase our understanding of this dynamic process as well as identify potentially new targets for vaccines and diagnostic reagents.

Grant: 1R01AI055916-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: KUSNER, DAVID J MD
Title: Inhibition of Macrophage Innate Immunity in Tuberculosis
Institution: UNIVERSITY OF IOWA IOWA CITY, IA
Project Period: 2003/08/15-2008/01/31

DESCRIPTION (provided by applicant): Tuberculosis (TB) is one of the world's greatest health problems, causing approximately 3 million deaths per year. Despite continuing increases in global morbidity and mortality, therapeutic and preventative options for TB remain severely limited. The central feature of TB pathogenesis is infection and intracellular survival of *Mycobacterium tuberculosis* (Mtb) within human macrophages. Following phagocytosis, Mtb evades the normally potent antimicrobial defenses of innate immunity by inhibiting the maturation of its phagosome to a microbicidal phagolysosome. The molecular mechanisms by which Mtb blocks phagosomal maturation and survives intracellularly are incompletely understood. The long-term goal of this project is to define the molecular mechanisms of tuberculous pathogenesis, to provide a foundation for improved therapies and vaccines. Recently, we demonstrated that live, virulent Mtb, but not killed Mtb, inhibit macrophage Ca^{2+} -signaling, and that this defect in host activation directly contributes to inhibition of phagosomal maturation and promotion of the bacilli's intracellular survival. Important gaps in our knowledge include: (1) the mycobacterial determinants responsible for inhibition of macrophage Ca^{2+} -signaling, and (2) the macrophage targets of Mtb-induced inhibition during this critical phase of the host-pathogen interaction. The hypotheses are: (a) sphingosine kinase (SK) is a critical target of macrophage deactivation by live Mtb, and (b) inhibition of SK is causally related to defective Ca^{2+} -signaling, inhibition of phagosome maturation, and the survival of Mtb within human macrophages. We will investigate these hypotheses by pursuing the following Specific Aims: (1) Characterize the activation of macrophage SK during phagocytosis of killed Mtb and its role in Ca^{2+} -signal transduction and phagosome maturation. (2) Determine whether inhibition of SK-mediated Ca^{2+} -signaling by live Mtb is causally related to defective phagosome maturation and intracellular viability. In Aims 1 and 2, pharmacological, biochemical, and genetic approaches will be used to modulate specific signaling pathways. (3) Determine the component(s) of Mtb responsible for inhibition of macrophage SK- and Ca^{2+} -mediated activation. A genetic approach of screening a transposon mutant library of Mtb and a biochemical approach of direct assessment of subcellular fractions of Mtb for effects on macrophage SK- and Ca^{2+} -mediated signal transduction will be undertaken.

Grant: 1R01AI055945-01
Program Director: KLEIN, DAVID L
Principal Investigator: MURPHY, JOHN R PHD
Title: Diphtheria & Anthrax Toxins: Mechanisms of Cell Entry
Institution: BOSTON MEDICAL CENTER BOSTON, MA
Project Period: 2003/07/01-2007/12/31

DESCRIPTION (provided by applicant): The catalytic domains of diphtheria toxin, anthrax toxins (protective antigen [PA], lethal factor [LF] and edema factor [EF]) and all serotypes of botulinum neurotoxin follow a similar route of entry into the cytosol of their respective targeted eukaryotic cells. Following binding of each toxin to their specific cell surface receptor, the toxin is internalized into the cell by receptor-mediated endocytosis in clathrin coated pits. Following acidification of an early endosomal compartment, the translocation of their respective catalytic domains to the cytosol is facilitated by at least a functional transmembrane domain. Using an in vitro translocation assay, we have recently demonstrated that a cellular translocation factor (CTF) complex is required for the delivery of the diphtheria toxin C-domain across the membrane of early endosomal vesicles. The X-ray structure of diphtheria, anthrax toxin, and botulinum toxin A is known. BLAST analysis of these toxins has suggested the presence of 12 amino acid "entry motif" in each toxin. The present application proposes the continuing study of diphtheria toxin C-domain, alanine scanning mutagenesis of the putative "entry motif", and the development of an in vitro translocation assay for anthrax lethal factor. In the case of diphtheria toxin, anthrax toxins, and botulinum toxins a great deal is known of their respective structure function domains and their mode of action; however, a detailed understanding of the molecular mechanism(s) required for translocation of their respective catalytic domains from the lumen of acidified early endosomes to the cytosol have remained elusive. Only a few studies have focused directly on the molecular mechanism(s) of translocation of the toxin catalytic domain entry have been published. In the case of diphtheria toxin, the results of these studies have led to two divergent hypothetical mechanisms of catalytic domain translocation. The first is based on the hypothesis that the toxin itself carries sufficient structural information to mediate translocation of its catalytic domain across the endocytic vesicle membrane. In contrast, the second hypothesis is based on findings that both structural domains of the toxin in combination with a cytosolic translocation factor (CTF) complex are necessary for productive translocation. We have used purified early endosomes charged with the fusion protein toxin, DAB3891L-2, to critically examine both hypotheses.

Grant: 1R01AI055962-01
Program Director: VAN DE VERG, LILLIAN L.
Principal Investigator: STARNBACH, MICHAEL N. PHD
Title: Inhibition of T cell Responses by Bacteria
Institution: HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA
Project Period: 2003/06/15-2007/11/30

DESCRIPTION (provided by applicant): We have initiated studies to characterize the role of CD8+ T cells in immunity to the intracellular bacterium, *Shigella flexneri*. During infection, *S. flexneri* enters cells and escapes into the host cell cytosol. We expected that proteins excreted from *Shigella* would be proteolytically degraded into peptides by the MHC-I processing pathway, and that the resulting cell-surface peptide:MHC-I complexes would stimulate CD8 + T-cells. During infection with *Listeria monocytogenes*, another cytosolic bacterial pathogen, *Listeria*-derived peptides, in complex with host MHC-I, are recognized by CD8+ T cells and contribute to the generation of protective immunity. However, we have found that CD8+ T cells do not appear to play a role in protective immunity to *S. flexneri*. Even when the *Shigella* have been engineered to constitutively secrete heterologous epitopes known to stimulate potent CD8 + T cell responses, those responses were not detected. We also found that when cultured cells were infected with these epitope-tagged *S. flexneri* strains, the cells were not recognized by established T-cell clones specific for the epitope tag. These findings have suggested to us that a step (or steps) in the normal MHC-I processing pathway is inhibited in cells infected with *S. flexneri*. The experiments in this proposal seek to identify and characterize the defect in MHC-I processing and/or presentation that occurs during *S. flexneri* infection. Specifically: 1) using biochemical assays, we will analyze the MHC-I pathway during *Shigella* infection to determine if there is inhibition of specific activities; and 2) we will use two parallel genetic screens to identify *S. flexneri* gene product(s) responsible for the inhibition. Through these experiments, we expect to identify and describe the activity of a bacterial inhibitor of MHC-I processing and presentation. Such an inhibitor might represent a novel class of virulence determinants specifically able to inhibit pathogen recognition by the adaptive immune system of the host. Understanding how this inhibition affects bacterial virulence and acquired immunity will further our understanding of the complex interaction of this bacterial pathogen and its mammalian host.

Grant: 1R01AI055987-01
Program Director: HALL, ROBERT H.
Principal Investigator: YILDIZ, HAVVA F BS
Title: Smooth to Rugose phase variation in *Vibrio cholerae*
Institution: UNIVERSITY OF CALIFORNIA SANTA CRUZ SANTA CRUZ, CA
Project Period: 2003/09/30-2008/01/31

DESCRIPTION (provided by applicant): Cholera is a global disease; endemic to Bangladesh, regions of South America, Africa and Australia, and also the Gulf Coast of the United States with the potential for epidemics in all aquatic environments. It is estimated that 120,000 people worldwide die from cholera annually. *V. cholerae* causes periodic, seasonal outbreaks in regions where it is an established member of the indigenous aquatic flora and this capacity is linked to its survival under diverse environmental conditions. *V. cholerae* switches its colonial morphology from smooth and translucent type to wrinkled and opaque type termed rugose variant when exposed to environmental stresses. We hypothesize that the phase variation mediated changes in population composition of *V. cholerae* can increase aquatic survival chances of the organism. The long term goal of this project is to understand how *V. cholerae* survives between epidemics by focusing on the molecular mechanism of smooth to rugose phase variation, its physiological consequences and its effect on the aquatic survival of the organism. Towards this goal, we will focus on the following specific aims: 1) determine and characterize the molecular basis of the smooth to rugose phase variation, 2) characterize the transcriptional network governing rugose specific gene expression and characterize the physiological and behavioral changes in the organism resulting from phase variation 3) elucidate the effects of diverse environmental parameters on the aquatic survival properties of the smooth and rugose variants and on the frequency of phase variation. Understanding how the smooth to rugose phase variation is contributing to persistence and survival of *V. cholerae* O1 El Tor in environmental aquatic habitats, and elucidation of the genes and processes regulating the phase variation will further our understanding of the aquatic life cycle of an important human pathogen. Results obtained from this study should lead to the development of molecular tools that can be used to identify transcripts or proteins that are predicted to provide better environmental fitness to the organism in natural aquatic habitats. This information will prove useful in the prediction and/or control of cholera epidemics. Smooth to rugose phase variation presents another challenge in public health since this process renders the organism resistant to oxidative stress and chlorine-mediated killing. Chlorination is used as a first line of defense against *V. cholerae* and many other waterborne pathogens. Understanding the mechanism and regulation of phase variation may aid in designing methods/inhibitors for modulating the frequency of phase variation and thus biocide resistance in *V. cholerae* and other aquatic pathogens.

Grant: 1R01AI056006-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: NIERMAN, WILLIAM C PHD
Title: Regulation of Burkholderia mallei virulence genes
Institution: INSTITUTE FOR GENOMIC RESEARCH ROCKVILLE, MD
Project Period: 2003/07/01-2005/12/31

DESCRIPTION (provided by the applicant): Burkholderia mallei, the etiologic agent of glanders, is an obligate parasite of horses, mules and donkeys, and is classified as a Category B agent by the Centers for Disease Control and Prevention (CDC). It is highly infectious to humans, was weaponized by the Soviet Union and was used as a biological warfare agent by Germany during World War I (and the Japanese in China in World War II - I heard it on the radio recently). The complete genome sequence of B. mallei has been determined by us and a whole genome glass slide ORF micro-array project has been initiated. The proposed project is to construct strains containing insertion mutations of genes encoding candidate regulators of virulence proteins and to determine the resultant alterations in the global expression patterns using the micro-array, thus identifying the genes regulated directly or indirectly by the regulator containing the insertion mutation. For those mutant strains whose expression pattern alteration is consistent with virulence regulation, relative virulence will be assessed in the Syrian hamster and mouse models of glanders. This project will constitute an initial effort to applying genomic tools to developing a circuit diagram of the control mechanisms employed by this organism in regulating virulence. It will provide critical information on virulence control and virulence mechanisms of B. mallei and will be crucial to the development of effective therapeutic interventions and vaccine strategies.

Grant: 1R01AI056056-01
Program Director: HALL, ROBERT H.
Principal Investigator: GULIG, PAUL A
Title: Molecular Pathogenesis of *Vibrio vulnificus*
Institution: UNIVERSITY OF FLORIDA GAINESVILLE, FL
Project Period: 2003/08/01-2008/01/31

DESCRIPTION (provided by applicant): *Vibrio vulnificus* is a gram-negative bacterium that causes fulminating diseases in susceptible humans: septicemia after ingestion of oysters and wound infection from seawater. The major predisposing factor is iron overload. The mortality rates for septicemia and wound infection are 77% and 15%, respectively, and patients can die within 24 hours of contact with the bacteria. *V. vulnificus* is highly invasive and replicates rapidly in host tissues, leading to high numbers of bacteria and extensive tissue damage. Our use of subcutaneously inoculated, iron dextran-treated mice revealed extensive tissue damage that resembles human disease and differentiated virulent clinical strains from less virulent oyster strains. Virulent *V. vulnificus* replicated extremely rapidly in the mice and were resistant to PMNs. Our hypothesis is that the rapid growth rate of *V. vulnificus* in host tissues and resistance to host phagocytic defenses enable the bacteria to reach high numbers and cause tissue damage with multifactorial toxins. Our preliminary results have enabled the dissection of each of these factors in the animal model using genetic tools in use by us. We propose to continue to use a molecular genetic approach to identify virulence factors of *V. vulnificus*. The specific aims are to: 1) Use a combination of signature-tagged mutagenesis (STM), PhoA fusion/insertion mutagenesis, and in vivo selection for complementation of naturally occurring attenuating mutations to identify virulence genes of *V. vulnificus*, 2) Use a marker plasmid with the iron dextran-treated mouse model to differentiate the effects of virulence genes on growth, killing, and damage, and use in vitro models to characterize the virulence phenotypes in more detail, and 3) Complete the molecular version of Koch's postulates for important virulence genes. In aim 1 we will primarily use STM to obtain mutations in genes involved with rapid growth or evasion of PMNs. We will use PhoA fusion/insertion mutagenesis to identify genes encoding secreted proteins. In aim 2 after differentiating effects of mutations on growth in versus killing by the host, we will characterize damage by examining histopathology and examine the interaction of vibrios with PMNs by infecting neutropenic mice. In vitro assays will involve analysis of auxotrophy, iron acquisition, complement resistance, PMN resistance, and cytotoxicity to cell culture. These studies will elucidate mechanisms of fulminating, invasive disease caused by *V. vulnificus* as related to rapid replication, evasion of defenses, and damage to host tissues.

Grant: 1R01AI056067-01
Program Director: SCHMITT, CLARE K.
Principal Investigator: KALMAN, DANIEL BA
Title: Role of tyrosine kinases in EPEC and EHEC pathogenesis
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 2003/09/01-2008/02/29

DESCRIPTION (provided by applicant): Enteropathogenic E.coli (EPEC) and enterohaemorrhagic E. coli (EHEC O157:H7) are deadly contaminants in food and water world wide causing diarrhea and death. Formation of actin-filled membrane "pedestals" (also called "A/E lesions") beneath EPEC and EHEC are essential for the development of disease. Tyrosine phosphorylation by an unidentified host cell kinase of a translocated bacterial virulence factor, called Tir, is essential for EPEC pedestal formation. Our preliminary evidence indicates (i) that the cellular kinase resembles Abl, but that other tyrosine kinases may also suffice, (ii) that Abl or a related kinase regulates both EPEC and EHEC pathogenesis, and (iii) that PD compounds, which inhibit Abl kinase and are being developed to treat cancer caused by dysregulated Abl, block pathogenic effects in vitro of EPEC and EHEC, and of *C. rodentium*, a related pathogen that forms A/E lesions in mice. The goal of Aim I is to identify redundant Abl-like kinases. The goal of Aim II is to determine how Abl kinase regulates formation of A/E lesions. Such information will prove essential to understanding the physiological mechanisms of A/E lesion formation, and will inform the design of even more specific inhibitors useful in treating not only bacterial infections but also cancers caused by dysregulated tyrosine kinases. The goal of Aim III is to establish the efficacy of PD inhibitors in mice infected with *C. rodentium*. Such experiments are an important prelude to clinical testing of drug efficacy in human patients infected with EPEC or EHEC. The need is apparent. EPEC and EHEC are classified by the NIAID as Category B pathogens. In developing countries, antibiotics and rehydration therapy are generally unavailable to treat EPEC infections, and a requirement for high patient compliance further reduces their efficacy when they are available. For EHEC, antibiotics exacerbate symptoms perhaps by lysing bacteria and releasing toxins and, in the United States, are contraindicated. Furthermore, drug-resistant EHEC strains have been documented. In this regard, because PD compounds affect the host and not the bacterium, selecting resistant strains with PD is far less likely than with conventional antibiotics or antimicrobial compounds.

Grant: 1R01AI056068-01
Program Director: SCHMITT, CLARE K.
Principal Investigator: MECSAS, JOAN C BA
Title: Yersinia Yops in an Animal Infection Model
Institution: TUFTS UNIVERSITY BOSTON BOSTON, MA
Project Period: 2003/07/01-2007/12/31

DESCRIPTION (provided by applicant): Pathogenic bacteria overcome host defenses to establish infections at mucosal surfaces and in mammalian tissues. Thus, understanding bacterial-host interactions that occur at these sites is critical to understanding host defenses and immunity. Pathogenic *Yersinia* spps, *Y. pseudotuberculosis*, *Y. pestis* and *Y. enterocolitica* all contain a virulence plasmid, pYV, that encodes components of a type III secretion apparatus as well as effector proteins, called Yops. The type III secretion system injects Yops into mammalian cells where they disrupt and/or alter mammalian cell function. Most Yops have multiple protein targets and biochemical activities in cultured cells. However, their cellular targets in an animal infection model are unknown. YopH and YopE are crucial virulence factors that allow *Yersinia* to colonize many tissues and cause disease. By studying mutants of YopH and YopE that are defective in acting on specific host proteins in an animal model system of infection, the protein targets that YopH and YopE must inactivate to enable *Yersinia* to colonize tissues and cause disease will be defined. In addition, the host cells that are injected with YopH and YopE during infection and the host cell types that kill yopH and yopE mutants in different tissues will be identified. Combined, this knowledge will reveal the host defenses targeted by YopH and YopE in different tissues and the specific activities of YopH and YopE needed to thwart these host defenses to enable *Yersinia* to colonize and cause disease. The long-term goals of this project are to understand the role of all Yops for *Yersinia* spp. to colonize and cause disease in animal tissues and to understand the host defenses that prevent, combat and/or contain *Yersinia* infection in tissues.

Grant: 1R01AI056073-01
Program Director: RUBIN, FRAN A.
Principal Investigator: RUBENS, CRAIG E MD CLINICAL MEDICAL SCIENCES, OTHER
Title: Role of a novel signal transduction pathway in GBS
Institution: CHILDREN'S HOSPITAL AND REG MEDICAL CTR SEATTLE, WA
Project Period: 2003/09/30-2008/01/31

DESCRIPTION (provided by applicant): The gram-positive pathogen *Streptococcus agalactiae* (group B streptococci, GBS) is the principal cause of human neonatal pneumonia, sepsis and meningitis. GBS is also an emerging pathogen of immunocompromised adults. We recently identified and characterized a novel eukaryotic-type serine/threonine protein kinase (Stk1) and its cognate phosphatase (Stp1) in GBS. Mutants of this signal transduction pathway exhibited pleiotropic effects on cell growth, virulence and segregation of GBS, indicating the importance of this pathway in the regulation of various cellular processes. In vitro phosphorylation studies revealed that these enzymes are essential for reversible phosphorylation of many GBS proteins. Using mass spectrometric analysis, we identified one of these targets as a anganese-dependent inorganic pyrophosphatase (PpaC). Pyrophosphatases are critical for regulation of biosynthetic reactions in the cell. Based on our results, we hypothesize that this signal transduction pathway and post-translational modification of its targets are crucial for normal cellular functions in GBS. A combination of molecular, biochemical and proteomic approaches will be used to elucidate the role of this signal transduction pathway and its physiological substrates in growth and virulence of GBS. In aim 1, we will identify and characterize the upstream and downstream targets of this pathway. We will utilize modern proteomic techniques such as liquid chromatography and mass spectrometry to identify other key targets of this signal transduction pathway. We will perform deletion analysis and protein cross-linking studies, to identify proteins that bind to and activate Stk1. In Aim 2, we will complete functional characterization of the identified physiological substrate of this signal transduction pathway, PpaC. In Aim 3, we will construct mutations in ppaC and a few other regulated targets of this pathway, identified in aim 1, to assess their role in growth and survival of GBS. We anticipate that some of these genes including ppaC will be essential for GBS growth. As mutants inessential genes are not viable, we will use the modern RNA interference technology to evaluate their role in GBS growth and survival. Collectively, these studies will determine the biological significance of this signal transduction pathway and lead to the identification of novel targets of GBS, which may provide insights into their potential as antimicrobial targets.

Grant: 1R01AI056075-01
Program Director: SCHMITT, CLARE K.
Principal Investigator: ECKMANN, LARS MD
Title: HOST DEFENSES AGAINST PATHOGENIC E. coli
Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA
Project Period: 2003/09/30-2007/12/31

DESCRIPTION (provided by applicant): Pathogenic (diarrheagenic) *Escherichia coli* are an important cause of food-borne disease in the U.S. and developing countries. Most pathogenic *E. coli* strains, e.g. EPEC and EHEC, cause mucosal inflammation and disease without deep invasion into the intestinal mucosa or systemic spread. Although much progress has been made in understanding the mechanisms by which pathogenic *E. coli* cause disease (e.g. toxins), relatively little is known about the host defenses against these pathogens. Based on the lack of deep tissue invasion, we hypothesize that host defenses that operate in the intestinal lumen or at the mucosal surface are key for controlling and eradicating infections with most strains of pathogenic *E. coli*. Our preliminary data strongly support this notion since we found that B cells are absolutely required for clearance of an EPEC strain in a mouse model of infection. The proposed studies will build on this observation and define the mechanisms of B cell-dependent host defense against pathogenic *E. coli*, using murine infection models. We will focus specifically on the importance of secretory antibodies as immune effectors of B cells. These studies on specific immune effector mechanisms will be complemented with functional investigations on the regulation of mucosal immune defenses against pathogenic *E. coli*, with a particular focus on the functions of the immunoregulatory cytokine, IL-6. In addition, we will begin to determine the importance of the intestinal epithelium in orchestrating immune defenses against pathogenic *E. coli*, as epithelial cells are a focal point of interaction between host and most strains of pathogenic *E. coli*. These experiments will focus particularly on the physiologic functions of the transcription factor, NF-kappaB, in the intestinal epithelium, using a novel murine model we have developed in which a key component of the NF-kB signaling pathway is deleted selectively from intestinal epithelial cells. The proposed studies have the following Specific Aims: AIM 1. To define the mechanisms of B cell-dependent host defense against pathogenic *E. coli*. AIM 2. To determine the functions of IL-6 in host defense against pathogenic *E. coli*. AIM 3. To define the importance of epithelial cell NF-kappaB in host defense against pathogenic *E. coli*. Together, these studies will provide significant new insights into the key host defenses by which the host can eradicate infection with pathogenic *E. coli*, thus providing an important basis for designing immunization strategies against these pathogens.

Grant: 1R01AI056124-01
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: DE MARS, ROBERT I PHD
MICROBIOLOGY:IMMUNOLOGY
GY
Title: Targeted Genetic Modifications of Chlamydia Trachomatis
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 2003/07/01-2005/06/30

DESCRIPTION (provided by applicant): Chlamydia trachomatis is the most common cause of bacterial sexually transmitted disease and of preventable blindness in humans. Antibiotics (not inexpensive) are used to control ongoing infections but common re-infection frequently causes serious pathology, e.g. pelvic inflammatory disease, infertility and blindness. Despite growing evidence of versatile human B cell and T cell immune responses to Ct antigens, there is little evidence of long-lasting protective immunity following infection; somehow, the organism evades repulse or elimination by the immune system. Current inability to genetically manipulate Ct has impeded analysis that might increase understanding of how Ct infections work and how the immune system might be better engaged in the management of Ct infection. The proposed work is aimed at developing a method of introducing planned genetic modifications into many targeted Ct genes following a three step work plan: (i) Demonstrate how to genetically transform Ct by means of homologous recombination between cloned Ct DNA that is transferred into Ct and chromosomal DNA of recipient Ct. A cloned mutant gyr A gene that renders Ct resistant to ofloxacin (OFX) will be transferred into sensitive Ct and resistant transformants will be isolated by selection with OFX. (ii) Demonstrate how to use the results of (i) to replace a normal Ct gene with a cloned mutant allele by the use of a model 'homologous recombination vector'(HRV). The same mutant gyr A gene used for (i) will be used, but transformants will be isolated by selection for a different, non Ct- derived 'selection marker' that is part of the HRV. Homologous recombination in Ct-derived parts of the HRV will incorporate the selection marker and closely linked Ct DNA into the Ct chromosome, thereby replacing the indigenous gene with the mutant trans-gene. (iii) Use the results of (ii) to create model knockout mutant Ct strains that can be studied in animal models. A knockout mutant allele of the folA gene will be used for this model because folA - deficient transformants that normally would be unviable can be isolated by supplementation of the culture medium with reduced folic acid. A multitude of mutation/function investigations that could be based on these model demonstrations includes the possible development of attenuated strains of Ct that might be useful protective vaccines. Pairs of mutants used in (i) - (iii) above will also be used to detect genetic recombination in mixedly infected human host cells. There is clinical evidence that such recombination occurs in humans and may contribute to Ct evasion of protective immune responses.

Grant: 1R01AI056177-01
Program Director: TAYLOR, KATHERINE A.
Principal Investigator: MC CLANE, BRUCE A
Title: Clostridium perfringens Type B-D Virulence Plasmids
Institution: UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PA
PITTSBURGH
Project Period: 2003/07/01-2007/12/31

DESCRIPTION (provided by applicant): Clostridium perfringens type B, C, and D isolates have significant medical, veterinary, and biodefense importance. Several toxins (e.g. select agent "B" list epsilon toxin and beta toxin) expressed by type B-D isolates are encoded by genes present on large plasmids. The long-term goal of this project is to fully understand how those little-studied plasmids contribute to the virulence of type B-D isolates. To start progressing towards this goal, the following specific aims will be pursued: 1) constructing isogenic single and double toxin knock-out mutants in type B-D backgrounds using insertional mutagenesis approaches, 2) comparing the virulence of these newly-constructed isogenic mutants against their parents (and complemented mutants strains); this will be accomplished using in vivo and in vitro approaches that will evaluate enteric virulence (intestinal loop models), systemic virulence (intravenous injections of culture supernatants) and effects of an intraduodenal challenge mimicking the entire disease spectrum (i.e., both enteric and systemic disease), 3) using Aim #1 toxin mutants, which will carry virulence plasmids tagged with antibiotic resistance determinants, in mixed mating experiments to evaluate whether type B-D virulence plasmids can transfer between C. perfringens isolates via conjugation, 4) conducting phenotypic/genotypic analyses to evaluate the diversity of these isolates; these studies will involve examining type B-D isolates to determine how much beta- and/or epsilon-toxin (as appropriate) they produce, testing whether those toxin expression differences are related to promoter differences, determining if some type B-D isolates produce beta- or epsilon-toxin variants (as appropriate) with altered biologic activities, and examining the diversity of type B-D plasmid genomes using pulsed-field gel electrophoresis and microarray approaches, and 5) investigating non-toxin virulence plasmid functions by insertional inactivation approaches; if Aim #3 confirms that type B-D virulence plasmids can transfer via conjugation, Aim #5 will initially target putative DNA transfer genes on these plasmids. These studies are expected to provide critical information for developing improved vaccines/therapeutics against type B-D infections and for developing molecular assays to subtype these isolates, as necessary for forensic investigations in the event that type B-D isolates are deliberately released during a bioterrorism event.

Grant: 1R01AI056231-01
Program Director: HALL, ROBERT H.
Principal Investigator: BENNETT, BRIAN PHD
Title: Substrate & Inhibitor Binding in Leucine Aminopeptidase
Institution: MEDICAL COLLEGE OF WISCONSIN MILWAUKEE, WI
Project Period: 2003/09/30-2008/01/31

DESCRIPTION (provided by applicant): The long term objective of the proposed program of study is to provide information that facilitates advances in the design of potent, molecular target-specific chemotherapeutic agents against cancers, HIV- and pathogenic bacterial infection. The secreted leucine aminopeptidase (VpAP) from *Vibrio proteolyticus* has high homology with a number of aminopeptidases from pathogenic vibronaceae and aeromonads, including *V. cholerae*. The aminopeptidase is implicated in infectivity of these bacteria. A hydrophobic pocket adjacent to the active site in VpAP has structural homology with such a pocket in mammalian functional homologues and may be the site of substrate recognition. The mammalian enzymes are the molecular targets for anti-tumor, immunomodulatory and anti-HIV infectivity drugs. The specific aims presented herein are designed to test the hypothesis that preferred substrates of the aminopeptidase from *Vibrio proteolyticus* are recognized by a substrate-binding patch adjacent to the catalytic metal-containing center. These aims are pertinent to substrate-binding-site engineering and inhibitor design relevant to human pathologies. Specific Aim 1: Demonstrate substrate and substrate analog binding to the hydrophobic patch of the prototypical aminopeptidase (VpAP) from *Vibrio proteolyticus*. EPR spectroscopy of spin labeled VpAP and spin labeled substrate analogs will be used to localize substrate binding in VpAP. Specific Aim 2: Determine the binding constants and kinetic parameters for inhibitors of VpAP and the kinetic parameters of analogous substrates. Distinct values for K_d and K_i for inhibitors will be obtained by EPR spectroscopy and steady state kinetics, respectively. Specific Aim 3: Obtain local structural information through EPR spectroscopy of complexes of VpAP with substrates and substrate analogs bound at the hydrophobic pocket. Structural information will be obtained from analysis of dipolar couplings between spin labels and paramagnetic transition ions in the active site. Specific Aim 4: Identify specific enzyme-substrate residue interactions (i.e. Sn-Pn) important for substrate binding, specificity and orientation. Systematic kinetic studies of hydrophobic site mutants will be carried out.

Grant: 1R01AI056254-01
Program Director: AULTMAN, KATHRYN S.
Principal Investigator: MUNSTERMANN, LEONARD E PHD
Title: Phylogeography of Verrucarum sand fly disease vectors
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 2003/07/15-2006/12/31

DESCRIPTION (provided by the applicant): Globally, the disfiguring, cutaneous form of leishmaniasis produces 20-40 million human cases and is transmitted exclusively by the bite of infected phlebotomine sand flies. Leishmaniasis is endemic throughout the New World tropics, and it is now increasing noticeably in rural, forested and suburban settings. A second sand fly-vectored disease, bartonellosis, is a scourge of the South American Andes with cases concentrated in Peru--it has a high fatality ratio >80% in untreated cases. The current proposal focuses on the 28 sand fly species of the Verrucarum group (in the genus Lutzomyia), which includes at least 17 known or suspected vectors of one or more of these diseases. The specific aims are as follows: (1) To compare the population genetic structure of 2 of the most widely distributed members of the Verrucarum group, Lutzomyia verrucarum (bartonellosis vector) and Lutzomyia serrana (probable cutaneous leishmaniasis vector), in order to detect cryptic species and evaluate the relative significance of geographic barriers and linear distance to genetic differentiation using variable mitochondrial gene sequences (cytb). (2) To investigate genetic relatedness among morphologically almost identical species (6 species of the s. townsendi and 2 species of the s. serrana) using total evidence approaches--DNA sequences, hybridization and isoenzymes. (3) To infer a molecular phylogeny for species of the Verrucarum group and related subgenera, and genera, based on comparison of mitochondrial (12SrDNA, COI) and nuclear gene sequences (18SrDNA, 28SrDNA, and ITS2). (4) To use the inferred phylogeny for verifying morphologically defined species, series, subgenera, and genera definitions, by testing their monophyly. (5) To deduce the evolutionary history of geographical and ecological associations of this group of taxa. (6) To develop molecular tools for a rapid identification of sand fly taxa. Resolution of these aims will provide insights into sand fly population genetic structure, and effects of geographic isolation. It will test if morphological classifications are justified by phylogenetic history. From the suite of genetic profiles generated, species identification and species authenticity will be facilitated. The phylogenetic relationships of a large and medically important group, Verrucarum, will be clarified. A broader perspective to the relationships among the nearly 400 species in the genus Lutzomyia will be established. A better understanding of the taxonomic and phylogenetic relationships provided by these analyses will lead directly to more realistic assessments of disease risk and new insights into the nuances of environmental effects on sand fly evolution.



Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Grant: 1R01AI056257-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: LAAL, SUMAN PHD
Title: Antibody Based Diagnosis for TB
Institution: NEW YORK UNIVERSITY SCHOOL OF MEDICINE NEW YORK, NY
Project Period: 2003/09/30-2008/01/31

DESCRIPTION (provided by applicant): Attempts to devise a serodiagnostic test for TB have been made for decades with disappointing results, primarily because most of the antigens evaluated are not relevant to the human immune response. During the last 7 years, we have characterized the humoral immune responses in TB patients at different stages of active disease. These studies form the basis for the research that is now needed to develop a serodiagnostic test for TB. Our studies clearly show that antigens chosen on the basis of their immunodominance during different stages of active TB, and in both HIV-infected and non-HIV TB patients, will provide the greatest opportunity to develop a successful diagnostic test. In this context, we have already identified approximately 12 proteins in culture filtrates of *M. tuberculosis* that elicit antibodies in patients with incipient pre-clinical TB, non-cavitary TB or cavitary TB. This subset of antigens also elicits antibodies in patients co-infected with HIV and TB. Two proteins of this subset have been cloned, and provide a serodiagnostic assay with the highest sensitivity and specificity that has yet been achieved with any antigens. In the current application, we propose to: (a) Identify the additional immunodominant antigens. This will be done both by proteomic and molecular approaches; (b) Clone the genes for these antigens into *E.coli* and evaluate the reactivity of the recombinant proteins with antibodies from TB patients at different stages of TB; (c) Map the immunodominant epitopes on these candidate proteins by a variety of strategies; (d) Identify the immunodominant peptides of these antigens that are recognized by antibodies from patients across the spectrum of TB; and finally (e) Select and evaluate the combinations of peptides that will be the basis of a low-cost, rapid, point-of-care diagnostic test for TB with high sensitivity and specificity.

Grant: 1R01AI056289-01

Program Director: VAN DE VERG, LILLIAN L.

Principal Investigator: CURTISS, ROY III PHD
MICROBIOLOGY:MICROBIO
OGY-UNSPEC

Title: Recombinant Attenuated Salmonella Vaccines for Humans

Institution: WASHINGTON UNIVERSITY ST LOUIS, MO

Project Period: 2003/07/01-2007/12/31

DESCRIPTION (provided by applicant): Of the 18.9 million annual deaths (1997) due to infectious diseases, about 7.5 million, in addition to significant morbidity, are the result of infections by *Salmonella typhi*, *S. paratyphi A*, hepatitis B virus (HBV), *Plasmodium falciparum*, *Streptococcus pneumoniae* and *Mycobacterium tuberculosis*. In the belief that improving health, nutrition and economic well-being (the later dependant on the first two) provides the best means to enhance the quality of life globally and thus reduces conditions that result in warlike and terrorist behavior, we propose a vaccine development program based on our recent technical developments in using recombinant attenuated *Salmonella* vaccines. Our objectives include: (a) to design, construct and evaluate an attenuated derivative of *S. paratyphi A* that will serve as an antigen delivery vector by exhibiting regulated delayed lysis within effector lymphoid tissues in the immunized individual to release hepatitis B virus (HBV) core particles encoding (i) HBV pre S1, pre S2 and T-cell epitopes as a preventative/therapeutic vaccine against HBV and (ii) *P. falciparum* circumsporozoite epitopes as a vaccine against malaria; (b) to construct and evaluate the contribution of strain background and the RpoS* phenotype on immunogenicity of a recombinant antigen expressed by attenuated *S. typhi* vaccine strains; and (c) to design, construct and evaluate recombinant attenuated *S. typhi* vaccines to express and deliver protective antigens specified by genetic information from (i) *S. pneumoniae* to prevent pneumococcal disease caused by strains with diverse capsular serotypes and (ii) *M. tuberculosis* as a preventative/therapeutic vaccine. The *S. paratyphi A* and *S. typhi* recombinant vaccines should also provide immunity to infection by *S. paratyphi A* and *S. typhi*. We will also develop our Master File, prepare and fully characterize candidate vaccine Master Seeds for stability and safety, prepare and submit protocols for IRB approvals, submit information necessary to obtain INDs, and perform any other work needed to arrange that the best candidate vaccines be clinically evaluated in human volunteers.

Grant: 1R01AI056305-01
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: NORGDARD, MICHAEL V
Title: Structural Analysis of Treponema pallidum Lipoproteins
Institution: UNIVERSITY OF TEXAS SW MED DALLAS, TX
CTR/DALLAS
Project Period: 2003/07/01-2007/12/31

DESCRIPTION (provided by applicant): Syphilis, caused by the spirochetal bacterium *Treponema pallidum*, continues to play prominently as a sexually transmitted disease. Syphilis pathogenesis also represents a paradigm of bacterial chronicity and immune evasion, but virtually nothing is known about how *T. pallidum* carries out these enigmatic processes. More specifically, the *T. pallidum* outer envelope, comprised of a cytoplasmic and outer membrane, must serve as both the physical and functional interface within the human host. Unfortunately, even though the *T. pallidum* genome has been sequenced, there remains a scarcity of information on the functions of *T. pallidum* membrane and membrane-associated proteins that likely contribute to the spirochete's complex parasitic strategy. Among the putative membrane proteins, *T. pallidum* is postulated to encode about 24-35 lipoproteins. Membrane lipoproteins of other bacteria subserve many important physiological roles and also have importance as virulence factors, modular components of ABC-type transporters, protective immune targets, and proinflammatory agonists that evoke robust innate immune responses. However, the functions of the treponemal lipoproteins remain essentially undefined. In a departure from more traditional approaches to *T. pallidum* research, the proposed study brings together a group of treponematologists, molecular biologists, protein biochemists, and structural biologists to address this important information gap in a novel way. The Specific Aims of this proposal are: (1) To clone and express in *E. coli* the lipoprotein genes of *T. pallidum*, with emphasis on expressing high quantities of each polypeptide as a nonacylated (soluble) fusion protein; (2) To purify to homogeneity each fusion protein and perform biophysical assessments of protein conformation; and (3) To obtain protein crystals suitable for X-ray diffraction and solve the three-dimensional structure for each crystallizable lipoprotein. Extensive preliminary data and progress in other structural biology initiatives support the timeliness and feasibility of this project; many state-of-the-art protein structural characterization techniques will enhance overall success. Finally, structural data will be used to formulate new testable hypotheses regarding potential function(s) of the lipoproteins, new avenues of investigation for *T. pallidum* membrane biology and syphilis pathogenesis that are sorely needed.

Grant: 1R01AI056404-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: ORTH-TAUSSIG, KIMBERLY A BS
Title: Biochemical Characterization of Yersinia Effector YopJ
Institution: UNIVERSITY OF TEXAS SW MED DALLAS, TX
CTR/DALLAS
Project Period: 2003/09/30-2008/01/31

DESCRIPTION (provided by applicant): The microbial pathogen *Yersinia pestis*, was the causal agent for the devastating plagues killing millions of people in Europe, India, China and even the United States. The pathogen persists in flea/rodent ecosystems on almost every continent and can cause a short but extremely painful death in untreated victims. This pathogen is a potential weapon that may be used for Biowarfare and therefore it is important to understand how this toxin works. *Yersinia*'s modus operandi includes evading phagocytosis, destroying the host defense system and inducing programmed cell death in the target host cell. Only six proteins called *Yersinia* outer proteins (Yops) encoded on a virulence plasmid efficiently carry out these activities; one of them, YopJ, both blocks cytokine production and promotes apoptosis in target host cells. YopJ, a 32kD protein, blocks all major signaling pathways involved in cytokine production including all the MAPK signaling pathways and the NFκB pathway. The family of YopJ proteins, composed of effectors from plant and animal pathogens, has structural homology to a clan of cysteine proteases that includes adenoviral proteases and the ubiquitin-like protein proteases. Consistent with the proposal that YopJ is a hydrolase, an intact catalytic site is required for YopJ to block the activation of the evolutionarily conserved super family of MAPK kinases (MKK). Based on these observations, we propose that YopJ uses a novel mechanism to disrupt a component of the signaling machinery that is required for the activity of all of the aforementioned kinase-driven signaling cascades. We propose three Specific Aims to investigate our hypothesis: (i) To characterize the target(s) of the *Yersinia* effector YopJ in the mammalian MAPK signaling pathway. (ii) To characterize the target(s) of the *Yersinia* effector YopJ in the NFκB signaling pathway. (iii) To characterize the hydrolytic activity of YopJ in vitro. Results from these studies will identify the targets of YopJ and characterize the inhibitory activity of YopJ that will lead to the understanding of an evolutionarily conserved mechanism of regulation that is necessary for intracellular transmission of signals. Discovery of this mechanism is essential for understanding how signaling pathways are regulated in both plants and animals.

Grant: 1R01AI056499-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: PULENDRAN, BALI PHD IMMUNOLOGY
Title: ANTHRAX TOXIN, DENDRITIC CELLS AND ADAPTIVE IMMUNITY
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 2003/09/01-2007/02/28

DESCRIPTION (provided by applicant): Anthrax poses a clear and present danger as an agent of biological terrorism. The major virulence factor of *Bacillus anthracis* is the anthrax toxin, which comprises 3 subunits: protective antigen (PA), edema factor (EF) and lethal factor (LF). LF and PA together form a toxin known as lethal toxin (LT), which appears to play exquisitely different immunomodulatory roles, depending on the dose of toxin used. At low concentrations, it cleaves components of the MAP-kinase pathway (MKK1,2 & 3), thereby rendering macrophages anergic to further stimulation by LPS. The consequences of this for adaptive immunity are not known. Moreover, the effects of LT on dendritic cells (DCs), the most efficient antigen-presenting cells in the body, are not known. At higher concentrations, LT elicits a radically different outcome - lysis of macrophages and systemic inflammation, followed by rapid death of the host. The reason(s) for these strikingly different effects are not known. These mechanisms and their pathophysiological relevance will be investigated in the following aims: Aim 1: To determine the effect of LT on routine DC function and adaptive immunity in vitro Sub-Aim 1a) Activation and viability of DCs from spleens, lungs and skin exposed to different concentrations of LT; Sub-Aim 1b) Stimulation of antigen-specific T cells by DCs cultured with different doses of LT; Sub-Aim 1c) Effect of LT on antigen-specific naive and memory T cells. Aim 2: To determine the effect of LT on murine DC function and adaptive immunity in vivo Sub-Aim 2a) Does injection of sub-lethal doses of LT impair DC activation in vivo? Sub Aim 2b) Does injection of sub-lethal doses of LT impair immune stimulatory capacity of DCs in vivo? Sub Aim 2c) To determine the principal cell types and inflammatory mediators of toxicity elicited by high doses of LT Aim 3: To determine the effect of LT on distinct human DC subsets and adaptive immunity in vitro Sub Aim 3b) Activation, viability and function of human monocyte-derived DCs cultured with LT, Sub-Aim 3b) Effect of LT on distinct human DC subsets. Aim 4: To determine the pathophysiological relevance of LT-induced suppression during *B.anthraxis* infection Sub Aim 4a) To determine the phenotype, function and microenvironmental localization of DCs in the lymphoid organs, various times after infection with LT-deficient or LT-sufficient strains of *B.anthraxis* Sterne; Sub Aim 4b) To determine whether LT suppresses immune function during a *B. anthracis* infection in macaques. Thus, the overall goal of this proposal is to acquire a deeper, mechanistic understanding of anthrax pathogenesis, and to use this knowledge to devise novel therapeutic modalities, which may be optimally effective at different stages of the infection.

Grant: 1R01AI057086-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: RUSSELL, DAVID G PHD PARASITOLOGY, OTH
Title: Cell Biology of Mycobacterium tuberculosis Infection
Institution: CORNELL UNIVERSITY ITHACA ITHACA, NY
Project Period: 2003/07/01-2007/12/31

DESCRIPTION (provided by applicant): The success of Mycobacterium tuberculosis, and other pathogenic mycobacteria species, lies in their ability to modulate their phagosome and prevent it from differentiating into an acidic, hydrolytically-competent compartment. The consensus in the field is that the bacterium arrests the normal maturation process leading to retention of the vacuole within the cell's recycling/sorting endosomal system. Although many studies detail the presence or absence of host molecules implicated in control of membrane fusion, the mechanism by which the bacterium modulates the phagosome remain to be determined. This proposal describes an integrated series of projects that will address this issue, place it in the context of the normal maturation process for phagosomes, and determine the consequences to the bacterium if it is delivered to the lysosome. The proposal addresses the following three aims: 1. Analysis of the lipid constituents of the phagosome membrane during maturation of IgG-bead and M. tuberculosis-containing phagosomes. The phosphorylation status of several membrane lipids changes during phagosome biogenesis and these alterations drive the association with the membrane fusion machinery in the cell. We intend to map these changes and correlate them with phenotypic differences in the pathogen-containing compartment. 2. Isolation and characterization of M. tuberculosis mutants defective in modulation of the phagosome. We have developed a genetic screen for such mutants and are currently characterizing the genetic defects that prevent modulation of the phagosome. These mutants should provide the tools that we need to determine how the bacterium regulates its phagosome. 3. Identification of lysosomal constituents that are bactericidal to M. tuberculosis. We have found that isolated lysosomes kill M. tuberculosis when added to bacterial cultures. Preliminary characterization of this fraction suggests that the activity co-purifies with cathepsins S and B. We intend to identify the active component and characterize the mechanism that leads to bacterial death.

Grant: 1R03AI054198-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: SULING, WILLIAM J PHD
Title: FolK, a Mycobacterium tuberculosis Drug Target
Institution: SOUTHERN RESEARCH INSTITUTE BIRMINGHAM, AL
Project Period: 2003/04/15-2005/03/31

DESCRIPTION (provided by applicant): The purpose of this pilot project is to investigate the enzyme 6-hydroxymethyl-7,8-dihydropterin pyrophosphokinase (FolK, HPPK, EC 2.7.6.3) as a target for intervention in disease caused by Mycobacterium tuberculosis (MTB). Unlike vertebrate cells, which acquire folates exogenously through active transport, MTB and many other bacteria must synthesize folate de novo. HPPK is an enzyme present early in the metabolic pathway for the synthesis of reduced folates from GTP. The absence of HPPK in the host makes this enzyme an attractive target for chemotherapy. Depletion of reduced folates through inhibition of this pathway leads to inhibition of DNA, RNA and protein synthesis. Comprehensive studies of the folate pathway in mycobacteria are lacking but genes coding for enzymes in the pathway have been identified through the Sanger Centre MTB genome sequencing project. A DNA sequence in the MTB genome database has been annotated as a probable folK coding for HPPK. For this pilot study, we propose to establish that the gene listed as Rv3606c codes for HPPK. Our objectives are to clone and express Rv3606c in Escherichia coli, and prove that the protein is functionally HPPK. We will also assess the essentiality of the gene by construction of HPPK-deficient MTB strains. This will be done in MTB by allelic exchange mutagenesis and a counterselection method based upon a mycobacterial thermosensitive origin of replication and toxicity of the sacB gene to MTB in the presence of sucrose. The results of this pilot study will enable us to better understand the biochemistry of folate metabolism in MTB. It will also provide purified HPPK for future drug discovery studies based upon structure-activity relationships, molecular modeling and crystallographic structure-based drug design.

Grant: 1R03AI054406-01
Program Director: GIOVANNI, MARIA Y.
Principal Investigator: ZHANG, LIXIN PHD
Title: Library on a Chip: Bacterial Strain Microarray
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): Bacterial species, in contrast to human and animals, can be highly diverse with diversity both in gene sequences and presence or absence of genetic material. Strains from the same species can differ in gene content (gene presence or absence) by as much as 20% (1,2). Studies routinely draw potential functional inferences from the distribution of genes among epidemiologically different collections, for example, diseased compared to commensal isolates. This is currently accomplished by membrane-based dot blot screenings, a low throughput process prone to inconsistency. We propose creating a bacterial library chip/array to replace the current approach. We will array the genomes of a library of bacterial strains on a single chip that then can be probed for the presence or absence of specific genes and for the allelic variations of the genes. This new form of microarray technology, library array, will allow rapid determination of the potential importance and function of genes or gene fragments and, ultimately, increase our understanding of bacterial pathogenesis and transmission. Specially, we will: Aim 1. Determine optimal chip fabrication, probe preparation, and array hybridization conditions A. Establish procedures for DNA sample preparation and treatment B. Establish procedures for array printing and array hybridization Aim 2. Optimize and standardize bacterial library chip production A. Determine optimal amounts of targets and probes B. Establish quality control and assurance C. Standardize data collection and normalization Aim 3. Create full-scale library chips for genetic screening A. Print a chip each with a library of genomic DNA from 1024 E. coli strains B. Test applications using bacterial library array chips

Grant: 1R03AI054411-01
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: LIPUMA, JOHN J MD
Title: Burkholderia sp: Identification of major clonal lineages
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2003/04/01-2005/03/31

DESCRIPTION: (provided by applicant): Certain bacterial species within the genus *Burkholderia* are capable of causing significant human infection. *B. mallei* and *B. pseudomallei*, the causative agents of glanders and melioidosis, respectively, have gained attention recently as agents of bioterrorism. Species within the *B. cepacia* complex (Bcc) are emerging nosocomial pathogens and are capable of causing life-threatening infection in persons with chronic granulomatous disease (CGD) or cystic fibrosis (CF), the most common inherited lethal disorder in Caucasians. In CF, respiratory tract infection by Bcc is generally refractory to antimicrobial therapy and a significant proportion of patients succumbs to rapidly progressive pulmonary deterioration and sepsis. Recent work indicates that some Bcc species are much more frequently involved in infection in CF than others. Furthermore, at a subspecies level, certain strains or clones have been identified that infect multiple CF patients, suggesting that they have an enhanced ability to infect or to be transmitted within this population. The goals of the proposed research are to (i) identify major clonal lineages of human disease-causing Bcc by genotyping an extensive collection of isolates recovered from CF sputum culture, and (ii) identify genes specific to these major clones by using subtractive hybridization methodology. In the short term, the genotyping analysis will have an immediate impact on efforts to optimize current infection control strategies in CF. The genotyping data are also a prerequisite to future outcomes studies that will seek to correlate strain type and clinical course of Bcc infection. The identification of genes specific to major clones will provide an immediate avenue to investigate the potential roles of these genes as virulence determinants in *Burkholderia*. The long-term objectives of this work are to characterize the mechanisms whereby these virulence determinants enhance the pathogenicity and/or transmissibility of Bcc in human infection. This will enable the development of novel strategies to prevent and treat Bcc infections, and will also provide valuable insights to mechanisms involved in transmission of other respiratory pathogens. A better understanding of the pathogenicity of Bcc species is also likely to provide timely information regarding the mechanisms of virulence and person-to-person transmission of the closely related species *B. mallei* and *B. pseudomallei*.

Grant: 1R03AI054435-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: BARBIERI, JOSEPH T
Title: Molecular properties of Yersinia YopT
Institution: MEDICAL COLLEGE OF WISCONSIN MILWAUKEE, WI
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): Yersinia pestis is a human pathogen with multifactorial virulence factors, which inhibit the host response to infection, allowing lethal growth within the host. Virulent strains of Y. pestis possess a virulence plasmid, which encodes the genes for a type-III apparatus and type-III cytotoxins. Type-III cytotoxins are injected directly into the host cell by the bacterium to elicit pathology, YopE, YopH, YopM, YpkA, YopJ, and YopT. YopT contributes to the inhibition of phagocytosis of professional phagocytes by modulating their actin cytoskeleton. While the molecular basis for the reorganization of the actin cytoskeleton is not clear, YopT appears to interact with Rho, a monomeric cellular GTPase. This goal of this proposal is to characterize structure-function properties of YopT. It is anticipated that YopT will possess features that are common to type-III cytotoxins, but will also possess features that are unique. These studies will provide new insights to neutralize Y. pestis during human infections and allow the PI to begin studies on the type-III cytotoxins produced by Y. pestis, using expertise in the study of both conventional bacterial toxins and type-III cytotoxins. While no longer a general health threat, Y. pestis is believed an agent that can be used in biological warfare or bioterrorist attacks, a class A pathogen. The ability of YopT to interfere with the phagocytic process of cells of the innate immune system is a key step in the infectious process of Y. pestis. Elucidation of the molecular and cellular action of YopT will provide insight to prevent early colonization of humans by this pathogen.

Grant: 1R03AI054463-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: LIANG, FANG-TING PHD
MICROBIOLOGY:MICROBL
PHYSIOLOGY
Title: Influence of Inflammation on B burgdorferi Adaptation
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by the applicant): *Borrelia burgdorferi*, the Lyme disease spirochete, is maintained within a complex enzootic life cycle involving the tick vector and the mammal. The spirochete adapts to these diverse environments, in part, by selective gene expression. Environmental cues such as temperature, pH and nutrients influence in vitro *B. burgdorferi* gene expression. Analysis of the gene expression of lipoproteins in the infected murine skin has defined two remarkably different microenvironments for *B. burgdorferi* adaptation: naive status and immune status. In the naive state, *B. burgdorferi* expresses 116 of its lipoprotein genes while only 34 of them are expressed during chronic infection. *B. burgdorferi* is able to invade almost all organs and tissues of the mammalian host and these diverse microenvironments may influence its lipoprotein gene expression. This pathogen causes murine carditis and arthritis that parallel two major manifestations of human Lyme disease. It is unknown whether inflammation influences *B. burgdorferi* adaptation. In this project, the tissue differential expression of lipoprotein genes and the influence of inflammation on lipoprotein gene expression of *B. burgdorferi* will be investigated using the murine model. To achieve these goals, the following specific aims will be completed. 1. To investigate the tissue differential expression of *B. burgdorferi* lipoprotein genes in the bladder, heart and joint during murine infection; 2. To examine the influence of inflammation on *B. burgdorferi* adaptation.

Grant: 1R03AI054491-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: DORMAN, SUSAN E MD
Title: Mycobacterium tuberculosis cell wall virulence factors
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 2003/06/01-2005/05/31

DESCRIPTION (provided by applicant): Tuberculosis (TB) kills approximately 3 million people per year. An important characteristic of *M. tuberculosis* is its ability to survive within human macrophages, and mycobacterial cell wall components are plausible mediators of this effect. However, little is known about the role of specific cell wall components in either mycobacterial survival within host macrophages or modulation of macrophage responses to mycobacterial infection. In our laboratory, the recent generation of a *M. tuberculosis* transposon-mediated mutant library provides a unique opportunity to systematically investigate these issues. Our overall objective is to understand the role of *M. tuberculosis* cell wall components in the pathogenesis of TB. Our hypothesis is that *M. tuberculosis* cell wall components modulate macrophage immune responses, and that alteration of the cell wall will affect macrophage responses to mycobacteria. The specific aim of this R03 proposal is to characterize human macrophage responses to a) infection with *M. tuberculosis* transposon-mediated mutants predicted to have alterations in the cell wall, and b) stimulation with cell wall fractions from those mutants. Mutants with disruptions in genes known or predicted to be involved in biosynthesis of sulfolipids, lipoarabinomannan, mycolic acids, and phthiocerol dimycocerosate will be used. Host cell cytokine production, chemokine production, apoptosis, IFN gamma-mediated responses, and toll-like receptor-mediated responses will be characterized, and mycobacterial survival and growth within macrophages will be determined. This research will lead to a better understanding of how mycobacterial cell wall components affect bacteria-host cell interactions, and will facilitate identification of new anti-tuberculosis drug targets and vaccine strategies. If cell wall components contribute to *M. tuberculosis*-induced immune suppression, then evaluation of appropriate mutant strains as vaccines would be warranted. These studies will also serve as a foundation for development of rapid in vitro screening systems to evaluate the effects of other *M. tuberculosis* genes or cellular components on targeted host immune responses. Finally, new information from the proposed self-contained pilot studies will serve as the foundation for future comprehensive dissection of macrophage response pathways affected by specific cell wall components.

Grant:	1R03AI054606-01	
Program Director:	SCHMITT, CLARE K.	
Principal Investigator:	KUEHN, META J	PHD OTHER AREAS
Title:	Detection of in vivo ETEC Vesicle Production	
Institution:	DUKE UNIVERSITY	DURHAM, NC
Project Period:	2003/03/01-2005/02/28	

DESCRIPTION (provided by applicant): All Escherichia coli make vesicles derived from their outer membrane. Whereas nonpathogens produce vesicles that are relatively harmless "empty shells", pathogens produce vesicles packaged with virulence proteins. The overall objective of this research is to understand the role pathogen-derived vesicles play in disease. Vesicles are likely made in the host by pathogens during colonization, however this has not been experimentally addressed. This proposal describes the study of in vivo production of toxic vesicles by enterotoxigenic E. coli (ETEC). ETEC is an important diarrheagenic pathogen in third world countries and can be fatal for children. We have developed methods to purify and characterize ETEC vesicles from culture supernatants. Lipid and protein composition analyses of purified ETEC vesicles point to the outer membrane as their origin. Physiologically active heat-labile enterotoxin (LT) is enriched in ETEC vesicles and is present both inside and bound to the outside of the vesicle. Vesicles can transfer virulence factors, such as LT, from gram-negative pathogens directly into host cells. LT on the surface of vesicles mediates binding and subsequent internalization of entire vesicles by gut epithelial cells. Most recently, we have discovered transposon insertion mutants of E. coli that have increased and decreased levels of vesicle production in vitro, demonstrating that vesicle production is governed by several genetic loci. In this proposal, sensitive, quantitative methods are detailed to detect and isolate vesicles produced in vivo. Further, the toxicity of ETEC vesicles will be assessed in an animal model. The toxicity of purified vesicles produced by ETEC grown in vitro will be compared with those produced by ETEC in vivo. The data resulting from these aims will answer key questions regarding the likely important in vivo function of pathogen-derived vesicles. The results will provide a strong foundation for further research regarding vesicle-mediated LT secretion and vesicle-mediated dissemination of virulence factors to host tissues.

Grant: 1R03AI054622-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: PETERS, WENDY PHD
Title: Role of Chemokines in Anthrax Pathogenesis
Institution: J. DAVID GLADSTONE INSTITUTES SAN FRANCISCO, CA
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): The recent deliberate dissemination of anthrax spores has revealed significant gaps in our knowledge of anthrax pathogenesis. In anthrax infection, as in other inflammatory diseases, the macrophage can play opposing roles. Macrophages serve to limit the infection by ingesting and eliminating the spores and vegetative bacilli, but they can also be a reservoir for replication and dissemination of the bacteria. In this grant we will use recently developed murine genetic models to clarify the role of the macrophage in anthrax pathogenesis. We will establish the time course of leukocyte migration to the lungs and draining mediastinal lymph nodes of mice infected with pulmonary anthrax, and we will identify the signals responsible for this recruitment. Previous work from our group has established that the monocyte chemoattractant protein (MCP) family of chemokines and their receptor, chemokine receptor 2 (CCR2), play pivotal roles in the migration of macrophages and dendritic cells to sites of inflammation, and that they are essential for host survival after infection with *Mycobacterium tuberculosis*. Unlike *M. tuberculosis*, a prominent feature of infection with *Bacillus anthracis* is the systemic effects produced by anthrax toxin (ATX) acting on the macrophages. Thus in the case of anthrax, it is unclear if impaired macrophage trafficking would be detrimental, or perhaps even beneficial to the host. We will take advantage of our CCR2^{-/-} mice to directly address these possibilities in a model of pulmonary anthrax. These experiments will reveal whether paradigms established for the pathogenesis of *M. tuberculosis* apply to *B. anthracis*. We will also compare the responses of CCR2^{-/-} and CCR2^{+/+} mice to lethal toxin a component of ATX. Finally, we will attempt to produce a mouse model of cutaneous anthrax infection. Completion of the specific aims of this pilot grant will establish the kinetics of leukocyte trafficking in pulmonary anthrax, will determine if chemokines such as MCP-1 play an important role in macrophage trafficking and host survival in pulmonary anthrax, and may provide a rationale for the use of chemokine/chemokine receptor antagonists in the treatment of anthrax.

Grant: 1R03AI054690-01
Program Director: ZOU, LANLING
Principal Investigator: DONNENBERG, MICHAEL S MD
Title: Type IV Pilins as Vaccines against Bioterrorism Threats
Institution: UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD
SCHOOL
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): Type IV pili are essential virulence factors for many Gram-negative bacterial pathogens and type IV pilin proteins have made effective vaccines for veterinary use. *Burkholderia mallei* and *B. pseudomallei*, the causative agents of glanders and melioidosis, respectively, represent significant biowarfare/bioterrorism threats. A search of the unfinished genomes of these microorganisms reveals that they contain identical genes for type IV pilin proteins. We plan to conduct pilot studies that will provide preliminary data for future studies of the use of the type IV pilin proteins of these organisms as vaccines. Our first specific aim is to purify soluble pilin protein, raise antisera, and test sera from animals sacrificed at various time periods after experimental glanders infection for antibody responses to pilin. Our second aim is to construct a strain of *B. mallei* that has a deletion in the pilin gene and test its ability to express pilin, pili and associated phenotypes in vitro. The data and reagents generated in these studies will be used for future studies to assess the role of the type IV pilus in experimental glanders infection and the protective efficacy of passive and active pilin immunization against glanders and melioidosis.

Grant: 1R03AI054788-01
Program Director: HALL, ROBERT H.
Principal Investigator: DIRITA, VICTOR J PHD MOLECULAR BIOLOG
OTHER
Title: Structure of ToxR/S and TcpP/H: Virulence Regulators
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): This proposal seeks to develop methods for overexpressing, purifying and crystallizing membrane-localized transcription control proteins from *Vibrio cholerae*, the agent of human cholera disease. ToxR and TcpP are bi-topic membrane proteins with cytoplasmic DNA binding/activation domains. They control expression of important virulence factors by virtue of their ability to activate expression of *toxT*, the activator of genes encoding cholera toxin and toxin-coregulated pilus. The DNA binding/activation domains of ToxR and TcpP are homologous to the winged Helix-Turn-Helix (w-HTH) family of proteins. The structures of other w-HTH domains has been solved, but the unusual membrane topology of ToxR and TcpP, and significant preliminary data aimed at determining how they recognize DNA and activate *toxT* transcription, compel an interest in solving their crystal structures when bound to operator DNA. Upon completion, this study will enable us to discriminate between distinct hypotheses for how ToxR and TcpP function. Along with determining the structure of ToxR and TcpP, of interest also is overexpression, purification and crystallization of the structures of membrane effector proteins required for the activity of each: ToxS in the case of ToxR, and TcpH in the case of TcpP. The specific aims of the proposal are as follows: (i.) To purify a 6-His tagged version of full length ToxR for crystallization and structural determination with and without its *toxT* promoter binding site or its binding site in the *ompU* promoter (a promoter activated by ToxR independently of TcpP) (ii.) To purify a 6-His tagged version of full length TcpP for crystallization and structural determination with and without its *toxT* promoter-binding site (iii.) To obtain structural data on co-crystals of ToxR and TcpP on the *toxT* promoter (to determine whether protein-protein interactions affect DNA binding) (iv.) To purify FLAG epitope- tagged versions of ToxS and TcpH for crystallization and structural determination alone or in a co-crystal with ToxR or TcpP

Grant: 1R03AI054794-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: SAVIOLA, BEATRICE J PHD
Title: Characterization of Acid Induced Promoters of M. tb
Institution: WESTERN UNIVERSITY OF HEALTH POMONA, CA
SCIENCES
Project Period: 2003/05/15-2005/04/30

DESCRIPTION (provided by applicant): Tuberculosis exacts an enormous burden in morbidity and mortality on the global population. With the advent of the HIV epidemic and multiple drug resistances, disease due to tuberculosis has increased leading to a need for research into the basic mechanisms of pathogenesis. The long-range goal of this proposal is to understand how *Mycobacterium tuberculosis*, the causative agent of tuberculosis, can sense environmental stresses, up regulate critical genes, and survive in the hostile environment of the host. When *M. tuberculosis* invades a host it infects human macrophages. *M. tuberculosis* can also be found within host granulomas that have been shown to have an acidic pH. The putative promoter regions of the *M. tuberculosis* genes *lipF* and *Rv0834c* have been identified to be upregulated in response to acidic pH. The specific aims of this proposal are to: 1) characterize the putative promoter regions of *lipF* and *Rv0834c* to better understand how acid induced promoters are regulated 2) determine if *lipF* and *Rv0834c* are required for mycobacteria to resist acidic stress and identify additional stresses which may upregulate these genes. The expectation is that this work will result in the characterization of acid responsive promoter regions and provide the groundwork for the eventual identification of a general mechanism by which *M. tuberculosis* can resist environmental stresses such as acidic stress. This work is significant because it will contribute to a greater understanding of mycobacterial stress response and ultimately survival and persistence in the host during infection.

Grant: 1R03AI054798-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: LEE, RICHARD E PHD CHEMISTRY
Title: Whole Cell NMR Studies of MYcobacteria
Institution: UNIVERSITY OF TENNESSEE HEALTH SCI MEMPHIS, TN
CTR
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): This proposal aims to use live, whole-cell, high resolution magic angle spinning nuclear magnetic resonance spectroscopy (HRMAS-NMR) as a novel, nondestructive technique to study carbohydrate and carbonaceous metabolism in mycobacteria. There is currently intense research in genomic and proteomic studies of mycobacteria. However, complementary methodologies to study globally, the carbonaceous metabolic products from genes or proteins has been lacking and it is this deficit in knowledge that this proposal aims to address. Firstly, it is proposed to assign the complex, live, whole-cell HRMAS-NMR spectra of *M. bovis* BCG using purified standard metabolites and multi-dimensional NMR techniques. Secondly, it is proposed that mycobacteria can adapt their carbonaceous metabolism in response to different physiological conditions. Thus HRMAS-NMR, will be used to study changes in mycobacterial carbonaceous metabolites, including the cell wall, under different physiological conditions: (i) physical conditions of growth such as different media, temperature and pH; (ii) growth phase; (iii) exposure to anti-mycobacterial agents, including those which target the mycobacterial cell wall; (iv) the effects of conditions which may mimic growth in vivo; (v) differences in the HRMAS-NMR spectra between different mycobacterial species. The central hypothesis is that whole cell HRMAS-NMR will be an excellent way to study these changes. Thirdly, using whole cell NMR, a novel study will be performed of the mycobacterial cell wall tertiary structure. Initially, it is hoped to locate the position of metabolites identified in this study in the cell ultra structure. Finally, we plan to initiate 3D-NMR studies and studies of 3-bond torsional coupling constants that will provide 3-dimensional, conformational information on the macromolecules identified. The findings from these studies will lead to a better understanding of mycobacterial carbohydrate and lipid physiology and hence, their role in the virulence and pathogenesis of the tubercle bacilli.

Grant: 1R03AI054970-01
Program Director: KLEIN, DAVID L
Principal Investigator: RELMAN, DAVID A
Title: Bordetella Response to Host Cues and Cell Signals
Institution: STANFORD UNIVERSITY STANFORD, CA
Project Period: 2003/07/01-2005/06/30

DESCRIPTION (provided by applicant): Bordetella pertussis is the causative agent of whooping cough, a disease that affects 20-40 million people each year, and that is increasingly prevalent in the United States. B. pertussis responds to environmental changes in the host airway by coordinately regulating the transcription of virulence factors, suggesting that virulence factor expression in vivo can be temporally and spatially restricted, thereby enabling efficient colonization and persistence of the pathogen. Our current understanding of relevant environmental cues and their corresponding B. pertussis gene expression responses is incomplete. The long-term objectives of this project are (1) to identify the environmental cues and signaling molecules in the human airway that influence B. pertussis gene expression and (2) to describe, at the genomic level, these B. pertussis transcriptional responses. Specifically, this proposal aims to 1) define B. pertussis transcriptional responses to components of the host environment including low concentrations of iron and amino acids, and presence of mucin, 2) measure B. pertussis global gene expression during host airway cell contact and intimate adherence, and 3) determine effects of growth phase and cell density on B. pertussis gene expression. These questions will be addressed at the genome level using a recently developed B. pertussis DNA microarray to simultaneously measure expression of almost every putative open reading frame. The knowledge gained from this study will lead to a detailed molecular understanding of B. pertussis virulence mechanisms and physiological adaptations, as well as the complex interplay between this pathogen and the human host, which could, in turn, lead to the development of novel pertussis vaccines and therapeutics.

Grant: 1R03AI054984-01
Program Director: GIOVANNI, MARIA Y.
Principal Investigator: NILSEN-HAMILTON, MARIT BS
Title: LPS-specific Aptamers for Microbial Detection
Institution: IOWA STATE UNIVERSITY AMES, IA
Project Period: 2003/05/01-2005/04/30

DESCRIPTION (provided by applicant): Lipopolysaccharides are a distinguishing feature of gram negative bacterial surfaces. Antibodies that recognize and distinguish between different O-specific oligosaccharide units are used to identify pathogenic bacterial strains that are been found in contaminated foods and have caused human death and disease. LPS molecules are often intimately involved in pathogenesis and participate in functions such as bacterial adhesion and movement, and in eliciting autoimmune responses. Septic shock syndrome, a severe inflammatory response that is often elicited by LPS, is deadly, killing 40% of individuals with symptoms. Recognition that pathogenic bacteria are deadly, easily dispersed and could be used for bioterrorism emphasizes the need to readily detect pathogenic bacteria and of having antidotes to protect against them. Antibodies are a very important means of detecting specific LPS molecules. However, the production and specificity of antibodies is expensive and results can be variable. On occasion, antibodies are also cross-reactive between two LPS structures or with carbohydrates in human tissues and thus have limited use. Here we propose to develop aptamers that recognize specific LPS structures. Aptamers are nucleic acids that, like antibodies, specifically recognize with high affinity the target macromolecules against which they were selected. Unlike antibodies, aptamers are selected ex-vivo and consequently can be more readily modified than antibodies so as to possess particular desired characteristics. Aptamers also possess many characteristics that are advantageous for diagnostics and field work. Our long term goal for anti-LPS aptamers is that they will add a new dimension to the ability to detect LPS from bacterial surfaces and that these aptamers also might find use in passive immunity or in other medical applications. We foresee the use of aptamers in homogeneous assays and in microarrays for on location detection of pathogens as a part of future in-place detection systems in public spaces for early detection of bioterrorist activities. The specific aims for this application are to: 1) Select for aptamers that bind LPS with high specificity and high affinity, 2) Characterize the selected aptamers, 3) Develop an allosteric aptamer CLAMP that detects a specific LPS oligosaccharide.

Grant: 1R03AI055285-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: WALMSLEY, AMANDA PHD
Title: A Plant-Derived, Multi-Component Tuberculosis Vaccine
Institution: ARIZONA STATE UNIVERSITY TEMPLE, AZ
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): Tuberculosis is the leading cause of death due to a single infectious agent among adults in the world. The HIV-AIDS pandemic has had a strong influence on the incidence of tuberculosis in the developing world, greatly increasing the annual risk of developing tuberculosis in HIV positive individuals and contributing to the spread of tuberculosis in the community. The current tuberculosis vaccine, bacillus Calmette-Guerin (BCG) has a variable protective efficacy and can cause serious, even fatal disease in immunocompromised patients. An effective, inexpensive, easily distributed and administered subunit vaccine is required for the control of tuberculosis. The goal of this project is to investigate the ability of plant-derived, tuberculosis antigens to induce immune responses appropriate for the protection of mice against tuberculosis. The success of this project will be the first step in our overall goal of developing an inexpensive, subunit vaccine that will enable control of tuberculosis (TB) in humans including immunocompromised individuals. Investigations will include the ability of plants to correctly process and express protective antigens against Mycobacterium tuberculosis; verification of the mucosal immunogenicity of the resulting plant-derived antigens; characterization of the induced immune response(s) in mice and guinea pigs and the ability of the plant-derived antigens to protect animals from challenge with M. tuberculosis. Synthetic, plant optimized coding regions have been constructed for the tuberculosis antigens Ag85B and ESAT-6. These synthetic coding regions have been fused to the B and A2 subunits of the heat labile enterotoxin (LT) of enterotoxigenic Escherichia coli (ETEC) to promote targeting to mucosal lymphoid tissues. The resulting fused coding sequences were cloned into plant expression vectors for future Agrobacterium-mediated transformation of tomato (Lycopersicon esculentum L. cv "TA234). Expression, correct folding and concentration of the antigens in plant materials will be verified through Western analysis and enzyme linked immunosorbent assays (ELISA). Plant lines displaying high antigen expression, or elite plants, will be cloned, and transferred to the TB Research Materials and Vaccine Testing Contract at Colorado State University to be tested for mucosal immunogenicity and protective ability in mice trials.

Grant: 1R03AI055545-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: SCHNEEWIND, OLAF MD
Title: The Type III Pathway of Yersinia Pestis
Institution: UNIVERSITY OF CHICAGO CHICAGO, IL
Project Period: 2003/07/01-2005/06/30

DESCRIPTION (provided by applicant): The human pathogen *Yersinia pestis*, as well as its close relatives *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*, employ a virulence plasmid-encoded type III secretion pathway to escape phagocytic killing during host infection. Type III secretion provides for *Y. pestis* multiplication and spread within lymphoid tissues as well as for the pathogenesis of acute lethal infections. The *Yersinia* type III pathway transports 13 polypeptides either into the extra-cellular medium (YopBDR and LcrV) or into the cytosol of host cells (YopEHMNOPT and LcrQ). During infection, *Yersinia* receive host-specific environmental cues that are transduced to relieve repression of the type III pathway. Several regulatory mechanisms of gene expression and type III transport have emerged. Moreover, the type III machinery represents a unique secretory system that recognizes its substrates via signals encoded in yop mRNA. The genes and mechanisms that are required for type III substrate recognition and regulation of gene expression or transport are still unknown. The virulence plasmid of *Y. pestis* encodes components of a type III secretion machine, its transport substrates and several regulatory factors. Preliminary studies show that the chromosome of yersiniae encodes additional genes involved in type III secretion. The purpose of this proposal is to exploit the *Y. pestis* genome sequence, the *Y. pestis* growth-restricted phenotype and the availability of mini-Mu transposable elements for random insertional mutagenesis, thereby isolating all *Y. pestis* genes that are required for type III secretion. Identified genes will be characterized further to reveal the mechanisms of substrate recognition, regulation of type III transport and pathogenesis of plague infections. Together these studies are also aimed at identifying molecular targets for drug therapies, an important area of research to aid in the defense against biological warfare and bioterrorist deployment of the select agent *Y. pestis*.

Grant: 1R03AI055575-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: FALL, R RAY PHD
Title: Phospholipid-induced Motility in Bacillus cereus Group
Institution: UNIVERSITY OF COLORADO AT BOULDER BOULDER, CO
Project Period: 2003/07/01-2005/06/30

DESCRIPTION (provided by applicant): The goal of the proposed research is to characterize newly-discovered gliding motility in bacteria of the Bacillus cereus group. The B. cereus group, which includes pathogens of humans (B. cereus and B. anthracis) and insects (B. thuringiensis), are quite variable in motility as measured on agar surfaces. We have found that B. cereus and B. thuringiensis exhibit very rapid phospholipid- or surfactant-induced gliding motility, independent of flagella and akin to the gliding/twitching motility seen in Gram-negative bacteria. Motility has been shown to be a key component of the pathogenicity of a variety of Gram-negative bacteria, and this may also be true for the B. cereus group. In B. thuringiensis we have also found that the phospholipid-induced motility is regulated by the plcR regulon, which is known to control the expression of numerous pathogenicity genes in B. thuringiensis as well as B. cereus. Our experimental plan, focusing on the B. cereus group, will be complemented by genome sequences of B. cereus and B. thuringiensis which are nearing completion. Specific aims include: (1) basic characterization of phospholipid-induced gliding motility in the B. cereus family; and (2) identification of genes essential for gliding motility in B. cereus and B. thuringiensis including analysis of the control of gliding by the plcR regulon. The work proposed here will provide fundamental, new information on the phenomenon of phospholipid-induced gliding motility in B. cereus and B. thuringiensis, identification of genes essential for gliding motility, and location of such genes in the B. cereus family genomes and comparison to other known bacterial genes. Such information will be the basis for future studies on the biochemical mechanism of gliding motility in B. cereus family, and may lead to new strategies to control tissue invasion by these pathogens.

Grant: 1R03AI055725-01
Program Director: KORPELA, JUKKA K.
Principal Investigator: DAVID, SUNIL A MD
Title: Novel Leads for the Therapy of Gram-Positive Sepsis
Institution: UNIVERSITY OF KANSAS LAWRENCE LAWRENCE, KS
Project Period: 2003/08/01-2005/07/31

DESCRIPTION (provided by applicant): Sepsis is the leading cause of mortality in the intensive care unit. A common and serious sequel of systemic bacterial infections, sepsis accounts for some 200,000 fatalities annually in the US alone, a figure higher than that attributable to AIDS and breast cancer combined. The pathogenesis of septic shock is a consequence of the host response to bacterial components. In the Gram-negative organism, lipopolysaccharide, found on the outer membrane, plays a major role in inducing the systemic inflammatory response that ultimately leads to the shock state. Although the clinical outcome of Gram-negative and Gram-positive infections are indistinguishable, until very recently, the causative factor in the Gram-positive organism was unknown. Compelling evidence now points to a major role for lipoteichoic acid, an integral component of the cell wall in Gram-positive organisms. In the course of our continuing efforts in identifying small-molecules that would specifically bind and neutralize Gram-negative lipopolysaccharide, we have found that certain classes of compounds also inhibit lethality in murine models of Gram-positive shock. In this proposal, we aim to initiate preliminary screening of select compounds based on the leads we have already obtained. We will first compare the biological activities of isolated, homogeneous, lipoteichoic acid and petidoglycan and verify that lipoteichoic acid is toxic on a molar basis comparable to that of reference lipopolysaccharide. Of the many classes of compounds screened in the past for anti-endotoxin activity, useful leads have been found in three classes: (1) the lipopolyamines (2) dendrimers, and (3) bis-amidines. Focused libraries of these compounds will be subjected to two levels of screening. In the primary screen, the inhibition of key cytokines induced by lipoteichoic acid in human peripheral blood mononuclear cells will be quantified. Active compounds will be subjected to a secondary screen in which upstream cellular events (cytokine mRNA transcription) will be examined. This is to demonstrate that inhibition of cytokine release, if observed, is attributable to the blockade of the cellular recognition of lipoteichoic acid.

Grant: 1R03AI055838-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: MISSIAKAS, DOMINIQUE PHD
Title: Genetic analysis of the S aureus secretory pathway
Institution: UNIVERSITY OF CHICAGO CHICAGO, IL
Project Period: 2003/03/01-2006/02/28

DESCRIPTION (provided by applicant): Human infections caused by Gram-positive bacteria present a serious therapeutic challenge due to the emergence of antibiotic-resistant strains. Of concern are infections with *S. aureus*, *S. epidermidis*, and *E. faecalis*, microorganisms that are the most common cause of bacterial disease in American hospitals. These Gram-positive human pathogens have acquired resistance mechanisms to virtually all known antibiotics. The development of novel targets for antimicrobial therapy is therefore urgently needed. We are investigating the protein translocation pathways across the cell wall envelope of Gram-positive bacteria as potential targets for antimicrobial therapy. Experiments described in this application aim at identifying *S. aureus* genes that are required for the secretion of hemolysins, lipase and nuclease, exotoxin that are known play important roles during infection. Two approaches will be used. By generating knockout mutations in the *prfA* genes of *S. aureus*, we will ask whether the encoded peptidyl-prolyl isomerases are essential for the secretion of folded polypeptides. By conducting a generalized transposon mutagenesis and scoring for defects in the secretion of exotoxins, we will ask whether we can identify all genes required for *S. aureus* secretion. The identified genes will be characterized for their role in the establishment of *S. aureus* infections using a mouse model of disease. If we can identify genes and gene products in the *S. aureus* secretory pathway that are involved in the establishment of disease, the gene product could represent a novel target for antimicrobial therapy.

Grant: 1R03AI056036-01
Program Director: TAYLOR, KATHERINE A.
Principal Investigator: HOOVER, TIMOTHY R PHD
Title: Acetone metabolism in *Helicobacter pylori*
Institution: UNIVERSITY OF GEORGIA ATHENS, GA
Project Period: 2003/06/01-2005/05/31

DESCRIPTION (provided by applicant): *Helicobacter pylori* is a major human pathogen that colonizes the gastric mucosa, leading to gastric inflammation that can progress to chronic gastritis, peptic ulcer, gastric cancer or mucosal-associated lymphoma. The ability of *H. pylori* to establish a chronic infection in the human stomach indicates that it is well adapted to acquire the nutrients it needs for growth in this unique environment. Complete genomic sequences for two unrelated *H. pylori* strains, 26695 and J99, have greatly aided the understanding of the physiology of this bacterium. Both sequenced strains were reported recently to have the genes for a potential acetone carboxylase, an enzyme that initiates the metabolism of acetone by converting it to acetoacetate. Acetone is produced in the body upon the spontaneous decarboxylation of acetoacetate, one of the ketone bodies produced by the liver and used as an energy source when glucose is not readily available. Ketones are always present in the blood, with up to 185 grams of ketone bodies produced per day by the liver of a healthy adult. This proposal will test the hypothesis that *H. pylori* utilizes acetone as an important energy source for the bacterium in the gastric mucosa. The first specific aim of the proposal is to verify that *H. pylori* has a functional acetone carboxylase by expressing the protein in *Escherichia coli*, purifying it, and examining its ability to catalyze the carboxylation of acetone. The second specific aim is to determine if this enzyme is needed by *H. pylori* to establish a chronic infection in the gastric mucosa. The operon encoding the *H. pylori* acetone carboxylase will be disrupted and the resulting mutant strain will be examined for its ability to colonize the stomachs of mice and Mongolian gerbils. The proposed studies will expand knowledge of metabolic pathways in *H. pylori*, which will lead to a better understanding of how this pathogen establishes infections in humans and may provide new strategies for the prevention or treatment of *H. pylori* infections.

Grant: 2R15AI047412-02
Program Director: VAN DE VERG, LILLIAN L.
Principal Investigator: PISTOLE, THOMAS G PHD
Title: Host Cell Recognition of Salmonella Typhimurium
Institution: UNIVERSITY OF NEW HAMPSHIRE DURHAM, NH
Project Period: 2000/05/01-2006/08/31

DESCRIPTION (provided by applicant): Salmonellosis continues to be a major infectious disease in both the United States and elsewhere. The overall goal of this project is to gain a better understanding of the early events that occur during Salmonella infections. The proposed studies focus on the initial interactions of salmonellae with host defense cells, specifically neutrophils and macrophages. The first objective is to determine whether structures found on the outer surface of Salmonella, known as porins, are involved in the recognition of this pathogen by human neutrophils. Porin-deficient mutants will be compared with their corresponding wildtype counterparts in their ability to adhere to and be internalized and killed by these neutrophils. Microbial attachment will be measured using flow cytometry and fluorescence microscopy and internalization and killing, by viability assays. The second objective is to determine whether neutrophils that have passed across a model intestinal epithelial cell layer are modified in their ability to recognize and to kill Salmonella. A model has been developed in which Salmonella initiate a series of events in the intestine that result in the migration of neutrophils into the lumen. The goal of this study is to determine whether these neutrophils exhibit an enhanced ability to detect and kill these bacterial pathogens. The third objective focuses on the ability of purified porins to block the attachment of Salmonella to host defense cells. Highly purified porins and porin-lipopolysaccharide complexes will be used in in vitro competition studies. Taken together, these studies are expected to provide a better understanding of the early cellular events in Salmonella infections.

Grant: 1R15AI051350-01A1
Program Director: PERDUE, SAMUEL S.
Principal Investigator: DUNLAP, NORMA K PHD
Title: Peptidomimetics of D-Ala-D-Ala as Novel Antibacterials
Institution: MIDDLE TENNESSEE STATE UNIVERSITY MURFREESBORO, TN
Project Period: 2003/06/01-2006/05/31

DESCRIPTION (provided by applicant): Although there are currently numerous antibacterial drugs on the market, many bacteria are becoming resistant to existing drugs, and the emergence of these drug-resistant microorganisms is a significant threat to public health. Virtually all classes of antibacterials in use have been circumvented to some extent by various resistance mechanisms and as a result there is a continual need for new structural classes of antibacterials. Penicillins are bacterial cell wall synthesis inhibitors and act by inhibition of Penicillin Binding Proteins (PBP's), also known as D-D-peptidases. The substrate for the D-D-peptidases is the cell wall peptidoglycan strand ending in D-alanine-D-alanine. However, penicillins and other drugs of that class were not designed to inhibit the D-D-peptidases. The objective of this application is to design and synthesize inhibitors of the D-D-peptidases as potential antibacterial drugs. Hydroxyethylene peptidomimetics of peptidic enzyme substrates such as the HIV protease substrate have been previously developed as drugs. A similar design concept should also apply to the D-D-peptidase substrate. The long-term objective of this project is to synthesize a series of peptidomimetics of the dipeptide D-alanine-D-alanine. These compounds will be tested for enzyme binding and for antibacterial activity. Various peptidomimetics have been designed and the syntheses of several have been initiated. Linear analogs of D-ala-D-ala containing a carboxylic acid will be synthesized, as will cyclopropyl analogs. Tetrazoles have been used successfully as bioisosteric replacements for the carboxylic acids in a number of drugs. An example is the angiotensin receptor antagonist Losartan. Along those lines, both linear and cyclopropyl analogs of D-ala-D-ala containing a tetrazole as a replacement for the carboxylic acid will be synthesized and tested for enzyme binding and antibacterial activity. The proposed compounds constitute an entirely new structural class of potential antibacterials and as such should possess activity against resistant organisms. This would have a significant impact in the ability to treat bacterial infections.

Grant: 1R15AI053062-01
Program Director: TAYLOR, KATHERINE A.
Principal Investigator: FORSYTH, MARK H PHD
Title: Signal Transduction Response in *Helicobacter pylori*
Institution: COLLEGE OF WILLIAM AND MARY WILLIAMSBURG, VA
Project Period: 2003/05/15-2006/04/30

DESCRIPTION (provided by applicant): Genome sequencing of numerous prokaryotes has revealed a tendency toward decreased genetic investment in signal transduction machinery in species that exist in restricted ecological niches. Bacterial signal transduction systems are typically comprised of a sensory histidine kinase protein and a cognate transcription factor, known as a response regulator. Bacterial species capable of growth in multiple environments have large numbers of signal transduction systems, as many as 63 separate systems. Bacteria that are restricted to a pathogenic lifestyle and have no known environmental reservoir possess many fewer systems to detect and respond to environmental changes. *Helicobacter pylori*, the etiologic agent of peptic ulcer disease as well as gastric adenocarcinoma, appears to inhabit exclusively the human gastric mucosa. Genome sequencing of this bacterium reveals only 4 signal transduction pathways. This implies a somewhat limited repertoire of signals to which this pathogen can respond. This proposal takes advantage of the complete genetic characterization of two distinct pathogenic strains of *H. pylori* to examine the role of one specific signal transduction system in the control of gene expression. The studies described here will facilitate the determination of signals in the gastric ecosystem that serve as cues for *H. pylori* to alter its gene expression pattern. This will be accomplished by identifying genes used in the adaptive response to these environmental signals. These studies will utilize *H. pylori* mutants which are "blinded" to an aspect of their environment due to the targeted destruction of genes encoding a sensory protein. Utilizing whole-genome transcriptional profiling by means of DNA microarrays, the regulatory events comprising the adaptive response in this gastric pathogen will be mapped and the genes of the adaptive response will be characterized. Determination of the identity of genes whose transcription is regulated will allow potential environmental cues to be inferred and then experimentally determined. Similarly, use of recombinant transcription factors (response regulators) will allow the isolation of critical DNA sequences necessary to accomplish regulation of gene expression. Understanding of the gastric environment, as perceived through the sensory apparatus of *H. pylori*, will allow a better understanding of *H. pylori* pathogenesis and a more rational design of interventions into the infectious process.

Grant: 1R15AI053112-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: NORRIS, PETER PHD
Title: Glycomimetics of *S. aureus* CP Aminosugars
Institution: YOUNGSTOWN STATE UNIVERSITY YOUNGSTOWN, OH
Project Period: 2003/06/01-2006/05/31

DESCRIPTION (provided by applicant): Antibiotic resistant bacteria are an immediate concern in the clinic and there is an obvious and growing need for new and more effective treatments. *Staphylococcus aureus* is one of the most worrying of these microorganisms since strains have developed that do not respond to even the most potent antibiotics currently available such as vancomycin. Since *S. aureus* produces a capsular polysaccharide (CP) to protect itself from phagocytosis, attacking the biochemical machinery that is used to create this polymeric coating should be a viable target for therapeutic intervention. The glycosyl transferase enzymes that build the polysaccharides have yet to be isolated therefore creating small molecule glycomimetics that might inhibit enzymatic activity will provide tools for enzyme isolation, as well as possible lead compounds for antibiotic treatment. The three amino sugars that make up the capsular polysaccharides of the most prevalent strains of *S. aureus* are N-acetyl-D-mannose uronic acid (D-ManAcA), N-acetyl-D-fucosamine and N-acetyl-L-fucosamine. Glycomimetics of each of these sugars will be produced. The specific goals of this proposal are: 1. Chemical synthesis of N-acetyl-D-mannose uronic acid N-glycosides from an azidodeoxy synthon; 2. Development of our dithiane approach to C-glycosides and C-disaccharides, especially focusing on D-ManAcA analogs; 3. Preparation of 1-deoxy iminosugar derivatives of D-ManAcA; 4. Formation of N-acetyl-D-fucosamine- and N-acetyl-L-fucosamine-derived nitroglycals that will serve as precursors to both N- and C-glycoside analogs of these compounds; 5. Studies towards the synthesis of 3-component potential inhibitors of the enzymes putatively used to form the 13-D-ManAcA- (1,4)- α -L-FucNAc linkage in *S. aureus* type 5 CP and the corresponding J3-DManAcA-(1,3)- α -L-FucNAc linkage in the type 8 CP.

Grant: 1R15AI053195-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: SAMUELS, D SCOTT PHD
Title: Outer surface protein gene expression in *B. burgdorferi*
Institution: UNIVERSITY OF MONTANA MISSOULA, MT
Project Period: 2003/05/15-2005/05/14

DESCRIPTION (provided by applicant): Lyme disease, the most common arthropod-borne disease in the United States, is caused by infection with the spirochete *Borrelia burgdorferi*. *B. burgdorferi* synthesizes several outer surface proteins (Osps), including OspA, OspB and OspC. OspA is the target in the Lyme disease vaccine and is thought to be involved in the binding of *B. burgdorferi* to the gut of its tick vector. OspC is thought to be a transmission or mammalian colonization factor and its synthesis is induced during tick feeding. The variation of OspA and OspB versus OspC is likely a means by which *B. burgdorferi* adapts to the different environments of the tick vector and mammalian host, and prepares for the environmental transition. Our hypothesis is that DNA supercoiling senses environmental signals and transduces them into an altered gene expression program in which ospC transcription is directly affected by DNA supercoiling. This project proposes to dissect, using molecular genetic and biochemical techniques, the regulation of outer surface protein gene expression. Gac and Hbb are two architectural DNA-binding proteins that alter DNA structure and supercoiling in *B. burgdorferi*. We will genetically assay the role of Gac and Hbb in ospC transcription by mutating the gac and hbb genes in *B. burgdorferi*. In addition, we will define cis-acting sequences by constructing ospC promoter mutants. We believe that we will be able to probe the mechanism of the variation in outer surface protein gene expression in *B. burgdorferi*, which will contribute to the understanding of the basic biology of this pathogen and can lead to improved diagnostic, prevention and treatment strategies.

Grant: 1R15AI054577-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: NELSON, DONALD J PHD
Title: MD Study of Anthrax Edema Factor:Calmodulin Complexes
Institution: CLARK UNIVERSITY (WORCESTER, MA) WORCESTER, MA
Project Period: 2003/05/01-2006/04/30

DESCRIPTION (provided by applicant): The long-term goal of this project is to understand the precise conformational and dynamic requirements for both effective binding of calmodulin (CaM) to the anthrax edema factor (EF) and the generation of a fully functional adenylyl cyclase active site. A more detailed understanding of the structure and dynamics of the complex between the edema factor, calmodulin and substrate analogs should facilitate future efforts at designing structure-based drugs for the treatment of anthrax infections. This application proposes to investigate, using the molecular dynamics simulation (MD) approach, a large number of EF-CaM complexes containing single-site mutations in EF which are already known to affect either CaM binding or adenylyl cyclase catalytic activity. The specific aims of this proposal are to: 1) Characterize the conformational and dynamic consequences of EF mutations which give rise to known defects in CaM binding to EF. MD simulation input files for EF-CaM complexes containing EF mutations, at the EF:CaM interface, known to affect either CaM binding or CaM-induced adenylyl cyclase activity (L523A, K25A, Q526A, V529A and D647A), will be generated, and then subjected to MD simulations using Insight II software. The subsequent analysis will focus on conformational and dynamic differences in the MD trajectories between wild-type and EF variant complexes. MD results will be correlated with kinetic data on the ability of these EF variants to be activated by CaM, in order to determine the requirements for most effective binding of CaM to EF. 2) Characterize the conformational and dynamic consequences of EF mutations which adversely affect EF adenylyl cyclase activity. MD simulation input files for EF:CaM complexes containing EF mutations, at the EF adenylyl cyclase active site, known to affect the CaM-induced adenylyl cyclase activity (K346R, K353R, K353A, H577N, H577D, N583A, N583Q, N583H, E588A, D50A and N639A) will be generated, and subsequently subjected to MD simulations. MD simulations will be performed, both in the absence and presence of bound substrate analog 3'-deoxy-ATP. Analysis of the resulting MD trajectories will focus on the conformational and dynamic differences between the wild-type and EF variant complexes. All results will be correlated with available enzyme kinetic data on the ability of these EF variants to be activated by CaM, to determine the precise requirements for the development of a fully-functional EF adenylyl cyclase.

Grant: 1R15AI055515-01
Program Director: TSENG, CHRISTOPHER K.
Principal Investigator: GOMEZ, FRANK A BS
Title: Capillary Electrophoresis: Bioanalytical Applications
Institution: CALIFORNIA STATE UNIVERSITY LOS LOS ANGELES, CA
ANGELES
Project Period: 2003/07/15-2005/06/30

DESCRIPTION (provided by applicant): The broad goals of this research are to develop capillary electrophoresis (CE) as an analytical technique to study the physicochemical parameters of the antibiotics vancomycin (Van), ristocetin (Ris), and teicoplanin (Tel). Its focus is on demonstrating principles and on developing useful bioanalytical procedures that can be applied to the analysis of receptor-ligand interactions and microscale reactions. The evolution of antibiotic resistant bacteria has made the study and development of Van-like antibiotics a critical area of research focus. Hence, studies probing the physicochemical parameters of these antibiotics via chemical modification are highly warranted. Two specific CE techniques will be utilized in this work: on-column microreactor techniques and affinity capillary electrophoresis (ACE). Techniques utilized in this study include chemical modification and characterization, chemical separation and identification of small molecules by CE utilizing both laser-induced fluorescence (LIF) and ultraviolet/visible (UV/VIS) detection schemes and high performance liquid chromatography (HPLC), and enzyme kinetics. The research will develop new bioanalytical techniques and will focus on examining small biomolecules involved in the prevention of disease and, hence, to the issue of public health. This understanding will strengthen the scientific base underlying the design, preparation, and application of CE towards a host of health-related problems. The specific aims of the research are to I. Utilize On-Column Microreactor Techniques and ACE in the Estimation of Binding Constants Between Antibiotics and Ligands. II. Determine the Charge and pI of Antibiotics Using On-Column Microreactor Techniques. III. Examine the Kinetics of Derivatization of Antibiotics Using Reactive Chemical Reagents. IV. Estimate Binding Constants of Linked Antibiotics to Peptides. V. Conduct Experimental Studies on D-Ala-D-Lac Terminus Peptide Binding to Van-Group Antibiotics. The proposed research will: Demonstrate the versatility of CE in determining physicochemical parameters of antibiotics; Demonstrate high-throughput derivatization of receptors and ligands coupled to ACE; Provide for rapid synthesis and accurate analysis of multiple drug targets simultaneously; Require reduced sample volumes compared to complementary analytical techniques, and; Reduce sample waste and disposal.

Grant: 1R15AI055521-01
Program Director: KORPELA, JUKKA K.
Principal Investigator: DAVIES, DAVID G PHD
Title: Dispersion of *Pseudomonas aeruginosa* biofilms
Institution: STATE UNIVERSITY NEW YORK BINGHAMTON, NY
BINGHAMTON
Project Period: 2003/09/30-2006/08/31

DESCRIPTION (provided by applicant): We have evidence that spent culture medium, when added to *Pseudomonas aeruginosa* biofilms, induces a physiologically mediated dispersion response. We hypothesize that during growth, *P. aeruginosa* strain PAO1 releases one or more factors into culture medium that are able to induce the dis-aggregation of biofilm cell clusters, dispersing free swimming bacteria into the bulk liquid medium. We further hypothesize that this compound can be isolated in pure form and identified, and that it will have a spectrum of activity including bacteria other than *P. aeruginosa*. Our goal is to characterize and manipulate the natural dispersion process of *P. aeruginosa* biofilms. The research plan of the current application sets forth a program to 1) test the activity of spent medium as an inducer of biofilm dispersion, 2) purify the inducer by chemical and physical extraction and separation methodologies and, 3) identify the inducer and initiate a characterization of the mode of action of the dispersion response. The potential commercial possibilities for a compound that can induce biofilm dispersion include enhanced treatment of biofilm infections, improved treatment of burns, improved removal of biofilms from surfaces, improved hygiene in hospitals, more effective cleaning products to improve health in the home, and more. The strong commercial potential of this project make it appropriate in the context of a developing biotechnology initiative such as exists at SUNY Binghamton. This project will also provide students with exposure to an exciting area of science that incorporates pure and applied research, biotechnology development, and considerable opportunity for interaction with the industrial community. The need to train students in the study and manipulation of biofilms has been widely recognized as basic to medical training in the coming years. Unfortunately, the opportunities for students to study in this burgeoning field are limited at the University level. The educational goal of the proposed work is to broaden the availability of training in the study of biofilms. The proposed work will incorporate undergraduate research training in biofilm science by involving a number of qualified undergraduates in carrying out significant aspects of the proposed research activity.

Grant: 1R15AI055544-01
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: JEMILOHUN, PHILIP F BS
Title: Flagellar antigens of *Trichomonas vaginalis*
Institution: UNIVERSITY OF ARKANSAS AT PINE BLUFF PINE BLUFF, AR
Project Period: 2003/07/01-2006/06/30

DESCRIPTION (provided by the applicant): Of all the sexually transmitted diseases, Trichomoniasis is the most common non-viral infection. An estimated 200 million people are infected annually all over the world, with greater than four million new cases per year in the United States alone. The agent of the disease, *Trichomonas vaginalis*, is a protozoan parasite with four anterior flagella and a recurrent flagellum. Trichomoniasis has recently been associated with severe complications such as development of cervical cancer, high probability of infection by human immunodeficiency virus and adverse pregnancy outcomes. In spite of the high incidence of trichomoniasis and the complications associated with the disease, the mechanisms of pathogenesis of the parasite are not well understood and some drug resistant strains of *T. vaginalis* are emerging. Therefore, there is urgent need for research in the area of subcellular structures of the parasite for; (a) adhesion molecules whose antisera will be of prophylactic value (b) discovery of new targets for anti-parasitic drugs (c) better understanding (immunobiology and functions) of the subcellular structures of the parasite. Our Preliminary Studies revealed several flagellar polypeptides that showed strong immuno-reactivity with human anti-Trichomoniasis sera in immunoblots. Furthermore, studies from different laboratories have shown that *T. vaginalis* cells are immunogenic in humans and laboratory animals. Based on these reports and our Preliminary Studies we hypothesize that flagellar proteins of the parasite are involved in adhesion of the parasite to the host cells. This hypothesis will be tested by isolating flagella followed by immunoprecipitation and analysis of flagellar antigens on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SOSPAGE). The flagellar antigens will be used to immunize rabbits and the rabbit antisera will be utilized to screen the parasite for adhesion inhibition. Flagellar antigens whose antisera show significant degree of adhesion inhibition will be considered to be flagellar adhesins. The role of the adhesins as ligands in the cytoadhesion of the parasite to host cells will be tuftly examined by adhesion/adhesion inhibition assays. The adhesins will be characterized to know if they are surface exposed and the biochemical make up of the reacting epitopes. It is anticipated that the results of these studies will yield new information about the roles of flagellar proteins in the pathogenesis of *T. vaginalis*.

Grant: 2R21AI019497-18
Program Director: KORPELA, JUKKA K.
Principal Investigator: MALAMY, MICHAEL H PHD
MICROBIOLOGY:MICROBIO
OGY-UNSPEC
Title: Genetic System to Study Virulence in Bacteroides
Institution: TUFTS UNIVERSITY BOSTON BOSTON, MA
Project Period: 1983/01/01-2004/04/30

DESCRIPTION (provided by applicant): This study will focus on factors that allow the obligate anaerobe *B.fragilis*, although a component of the normal colonic microbiota, to be a successful pathogen. These include its ability to withstand an aerobic environment (aero-tolerance) during early stages of infection; the presence of systems to import heme into the cell for the heme-dependent pathways of central metabolism and defense against reactive oxygen species; the ability of *B.fragilis* to remove sialic acid residues from host components, and its virtuosity in obtaining nutrients for growth in vivo from complex oligosaccharides and glycoproteins. Specific aims include: 1, to continue to study factors that allow *B.fragilis* to withstand prolonged oxygen challenge (aerotolerance): We propose that activities in the *B.fragilis* periplasm serve as the initial line of defense to combat the formation of reactive oxygen species (ROS), protect sensitive targets from ROS challenges and to reverse ROS damage. In addition we have identified specific functions (superoxide dismutase, SOD), and an extensive gene cluster (the Bat operon) that are required for aerotolerance. We will test the hypothesis that the Bat operon plays an important role in exporting reducing potential from the cytoplasm to the periplasm. 2. Acquisition of iron and heme is important for *B.fragilis* growth in vitro and in vivo. We will study the process of heme uptake in *B.fragilis* by the heme permease systems whose genes and functions we have described. We will also continue to study the heme-dependent, and Fe-S cluster-containing enzymes in the dual pathways of central metabolism to establish their roles in aerotolerance and in providing energy during oxygen challenge. 3. to investigate the composition, functions and control of operons for the acquisition of growth substrates from the infected host. We will focus on the operon containing the neuraminidase (nanH1) gene and several other glycohydrolases capable of converting the complex Lewis antigen found on the surface of many human cells to individual monosaccharides. We will continue to define the operon for NANA utilization, the NanL1 operon. Is neuraminidase a virulence factor because it supplies NANA for *B.fragilis* growth, or because its activity alters the surface of host cells during infection, or both? 4. We will develop additional tools to define changes in macromolecular synthesis and stability of DNA, proteins and membranes during 9 oxygen challenge.

Grant: 2R21AI031088-10A1
Program Director: KLEIN, DAVID L
Principal Investigator: ARMSTRONG, SANDRA K PHD
Title: Iron acquisition in *Bordetella pertussis*
Institution: UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN
Project Period: 1991/08/01-2005/01/31

DESCRIPTION (provided by applicant): *Bordetella pertussis* is a bacterial pathogen that colonizes the human respiratory epithelium to cause whooping cough. To obtain nutritional iron, *B. pertussis* produces the dihydroxamate siderophore alcaligin and also expresses activities required for the utilization of host heme compounds as well as certain non-native siderophores, including the potent and ubiquitous catechol siderophore enterobactin. Genes encoding these three iron-scavenging systems have distinct positive transcriptional regulators that respond to the cognate iron source for maximal expression of the genes required for its utilization. Expression of all three positive regulators is repressed by the global iron starvation regulator Fur, using iron as corepressor. We hypothesize that *Bordetella* cells establish priorities in iron transport gene expression by sensing and responding to the presence of the available iron source and selectively activating expression of genes involved in its assimilation. The ability of *Bordetella* species to prioritize the expression of different iron systems may be important for effective adaptation and multiplication in the host environment. The proposed studies will analyze the *in vivo* importance of each of the three iron uptake systems in animal models of infection and will determine whether each system is functionally distinct or whether they have shared functional relationships. Mechanistic features of siderophore signaling and transcriptional activation will be delineated for the native alcaligin siderophore system and the enterobactin siderophore utilization system. The importance of the ability to transcriptionally respond to the appropriate iron source *in vivo* will be evaluated using *Bordetella* mutants producing novel hybrid regulators with reversed inducer and target gene specificities. A cell surface signaling phenomenon uniquely involved in regulation of the *Bordetella* host heme-iron utilization system will be investigated, and interacting signaling and regulatory protein domains will be defined. Spatiotemporal analysis of *in vivo* expression of the three iron systems will determine which systems are operational in the animal host and assess whether the systems are differentially expressed in certain tissue sites or during distinct stages of infection. Because *B. pertussis* is an obligate human pathogen with no known environmental or nonhuman animal reservoirs, it represents an ideal model organism for analysis of the host-parasite relationship and the physical, chemical and innate biological conditions that impact on the growth of bacteria in a host environment.

Grant: 2R21AI040915-06
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: RAULSTON, JANE E PHD
Title: CHLAMYDIA TRACHOMATIS ENVELOPE COMPONENTS AND VIRULENCE
Institution: EAST TENNESSEE STATE UNIVERSITY JOHNSON CITY, TN
Project Period: 2003/09/30-2004/09/29

DESCRIPTION (provided by applicant): Chlamydia trachomatis is the leading bacterial agent of sexually transmitted infections in the United States and a major culprit in urethritis, cervicitis, endometritis, salpingitis, pelvic inflammatory disease, infertility and ectopic pregnancy. The highest chlamydial infection rates are observed in young people between 15 and 34 years of age. Throughout these peak reproductive years, the endometrial epithelial cell layer lining the uterine cavity is subject to constant changes in levels of micronutrients such as iron, due to hormonal cycling during menstruation. Endometrial epithelial cells are natural target host cells for infection by chlamydiae. The availability of iron is well-known to have a tremendous influence on the production of bacterial antigens, envelope components and virulence factors; these effects are particularly prominent for obligate intracellular pathogens such as chlamydiae. In other pathogens, virulence factors produced in response to low concentrations of iron elicit tissue damage in the host. Certain bacterial iron-regulated proteins are also immunotherapeutic targets for vaccine design. In these studies, the mechanism for regulation of chlamydial iron-responsive proteins and antigens will be examined in Specific Aim 1. Specific Aims 2 and 3 will (i) determine the identities of chlamydial iron-regulated proteins, and (ii) quantitatively assess transcription of the genes encoding these components under iron-deficient growth conditions, respectively. In Specific Aim 4, an envelope transport system will be examined to determine whether or not it functions as a major iron-uptake pathway for the chlamydiae. The long-term objectives for these studies are to develop a better understanding of mechanisms for the destructive tissue pathology observed in chlamydial infections and to provide new insights on specific chlamydial proteins and antigens that could be tested for their immunotherapeutic potential.

Grant: 2R21AI042287-05A1
Program Director: VAN DE VERG, LILLIAN L.
Principal Investigator: SOLOSKI, MARK J
Title: T CELL RESPONSES TO INTRACELLULAR BACTERIAL PATHOGENS
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 1998/08/01-2004/06/30

DESCRIPTION (provided by applicant): In the last several years, significant progress has been made in understanding the relevance of the adaptive T cell immune response to the clearance of the Gram-negative pathogen *Salmonella typhimurium*. Studies in our laboratory established that CD8 + T cells are essential elements in the protective immune response against infection with *S. typhimurium*, and these studies led to the identification of peptide epitopes recognized by bacteria-specific CD8 effector T cells. In this revised competitive renewal, we will build on these initial observations and develop a model system where infection is initiated by the natural oral route of infection. This mode will allow us to define the essential cellular elements of the mucosal immune system that respond to also enable us to understand the contribution of innate cellular immunity to the clearance of infection. In the next five years, we will focus our efforts on the following Aims: Aim 1. What are the characteristics of the novel CD8 α /13 expressing Intraepithelial Lymphocytes (IELs) that are induced in the small intestine after oral infection with *S. typhimurium*? Specifically, we will determine the activation state, TCR usage, recognition properties and functional capacity of these T cell subsets. Aim 2. Do NK and NKT cells contribute to the development of a protective T cell-mediated immune response to *S. typhimurium*? Aim 3. Do bacteria, that display defined differences in cellular tropism (e.g., dendritic cells vs. epithelial cells), vary in their ability to stimulate both innate and adaptive host immune responses? The studies contained in this proposal are designed to address the various host immune elements that contribute to the clearance of infection and the generation of protective immunity to a model Gram-negative pathogen *Salmonella typhimurium*. We hope to apply this information to the design of vaccine strategies that will evoke potent protective immunity as well as contribute to understanding the etiological link between infection with gram-negative pathogens in the development of autoimmune disease. Given that many of the cellular receptors in the mouse model have human counterparts, we argue that this murine model will yield valuable information that may be applied to the human setting.

Grant: 2R21AI043268-06
Program Director: LAUGHON, BARBARA E.
Principal Investigator: JACOBS, WILLIAM R PHD
Title: INH-induced lysis of Mycobacterium tuberculosis
Institution: YESHIVA UNIVERSITY BRONX, NY
Project Period: 1998/07/01-2004/04/30

DESCRIPTION (provided by applicant): Mycobacterium tuberculosis is one of the most important opportunistic pathogens of HIV-infected individuals. Current treatments for tuberculosis are being threatened by the rapid emergence of drug resistance. Our research program has worked to define the mechanisms of action of a leading anti-tuberculosis drug isoniazid (INH) and an important second line drug ethionamide (ETH), with the goal of enabling rational drug design. Using a combination of genetics, biochemistry, X-ray crystallography, electron microscopic and gene analysis approaches, we discovered a common target to be an enoyl reductase of the Fatty Acid Synthase (FAS) type II system responsible for mycolic acid synthesis. The three dimensional structure of InhA was determined, and its enzymatic activity was unexpectedly found to be inhibited by an INH-NAD adduct that bound to the InhA NADH binding pocket. We have also identified a set of contiguous genes iniB, iniA, and iniC, that confer tolerance to INH in mycobacteria overexpressing these genes. We have established InhA as an excellent drug target by demonstrating that InhA-thermal inactivation of a temperature-sensitive mutation in inhA leads to lysis of the mycobacterial cell. Moreover, we have identified novel compounds that inhibit InhA and possess anti-mycobacterial activity. Despite these accomplishments, additional questions remain concerning the molecular events that lead to INH-induced cell lysis, and the mechanisms that confer resistance to this phenomenon. Here we propose to continue our multi-disciplinary approach to study novel resistance mechanisms, including one that is caused by defects in NADH dehydrogenase. We will also focus on defining the molecular events that lead to mycobacterial cell lysis by comparing and contrasting three different mutant strains that cause lysis following inhibition of three different metabolic pathways. Finally, we will characterize the tolerance mediated by the IniA and IniB proteins, and define the mechanisms by which mycobacteria prevent lysis. The knowledge gained by this work will lead to the identification of novel drug targets, strategies to overcome tolerance, and more effective treatments for tuberculosis.

Grant: 2R21AI043940-05
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: ALDERETE, JOHN F PHD
MICROBIOLOGY:MICROBIO
OGY-UNSPEC
Title: Trichomonas vaginalis Genetic Analysis of Cell Adherence
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR SAN SAN ANTONIO, TX
ANT
Project Period: 2003/09/01-2005/08/31

DESCRIPTION (provided by applicant): Trichomonas vaginalis is responsible for serious health consequences for women. Significantly, the infection by this parasite is a co-factor in amplifying the transmission of HIV among African American and Hispanic women contributing to poor minority health and health disparities in our nation. Fundamental aspects of Trichomonas cell and molecular biology remain unknown, despite the impact to public health. Our long-term goal is to understand the molecular basis of pathogenesis. Adherence of trichomonads to vaginal epithelial cells is important for infection, and, therefore, the objective of this proposal is to study the structure, function and regulation of T. vaginalis AP65 adhesin compartmentalization. The rationale for undertaking these studies is that more effective means of control of infectious diseases requires enhanced knowledge of the fundamental biological processes that govern virulence. The AP65 adhesin has sequence identity to malic enzyme (decarboxylating), an enzyme of organelles called hydrogenosomes. Thus, AP65 belongs to the category of surface-associated microbial enzymes with functional diversity. The hypotheses being tested are that i) iron regulates gene expression, compartmentalization and surface placement of all members of the ap65 gene family, ii) there is cross signaling by phosphorylation of trichomonads and AP65 following adherence, and iii) there is a quantitative relationship between host epithelial cell receptors for AP65 and levels of cytoadherence. Four aims are proposed. Aim 1 will show compartmentalization outside hydrogenosomes by expressing tagged-AP65 fusion in wild type and MR100 (AP65-negative) mutants. Aim 2 will determine the mechanism of compartmentalization of AP65. Aim 3 will examine the differential binding of trichomonads to host epithelial cells mediated through AP65 and cell receptor. Finally, Aim 4 will silence expression of the ap65 genes. In addition to the biological merit, innovative aspects of our proposal are our use of antisense transfection to silence expression of ap65, and the use of drug resistant MR100 lacking hydrogenosome proteins (AP65-negative) to study adherence.

Grant: 2R21AI044639-05
Program Director: LAUGHON, BARBARA E.
Principal Investigator: TONGE, PETER J PHD
Title: Inhibition of Fatty Acid Biosynthesis in M. Tuberculosis
Institution: STATE UNIVERSITY NEW YORK STONY STONY BROOK, NY
BROOK
Project Period: 1999/05/01-2005/01/14

The long term goal of this proposal is to generate novel inhibitors of fatty acid biosynthesis in Mycobacterium tuberculosis. It is hypothesized that such compounds will have antimycobacterial activity and will provide a appropriate starting point for generating drugs to treat multi-drug resistant tuberculosis. The proposal has two Aims. Specific Aim 1 focuses on the design and synthesis of inhibitors that target InhA, the enoyl reductase FASII enzyme. This enzyme is one of the putative targets for isoniazid, a frontline antituberculosis drug. Novel compounds will be synthesized based on the diphenyl ether skeleton of triclosan, an inhibitor of enoyl reductases in M. tuberculosis and other bacteria. Inhibitor design will utilize X-ray crystallography, Raman spectroscopy and computational approaches. Compounds will be tested using enzyme kinetics, the antimycobacterial activity will be assessed using MICs and the intracellular mode of action of the compounds will be evaluated using DNA microarrays and photoaffinity labeling. Specific Aim 2 will investigate the mechanism of action of isoniazid and will test the hypothesis that proteinprotein interactions within the mycobacterium modulate the sensitivity of InhA and other FASII enzymes to isoniazid. The FASII enzyme complex from M. tuberculosis will be purified and the activity and sensitivity of each enzyme component toward FAS inhibitors will be evaluated. Characterization of the FASII complex will reveal the identity of the dehydrase enzyme and the FASII complex will be reconstituted in vitro using recombinantly expressed proteins. In addition, pull-down experiments will be used to identify other InhA protein binding partners and characterization of the FASI enzyme complex from M. tuberculosis will be initiated.

Grant: 2R21AI049174-04
Program Director: LAUGHON, BARBARA E.
Principal Investigator: FAHEY, ROBERT C BS
Title: MYCOTHIOL BIOSYNTHESIS AND METABOLISM AS TB DRUG TARGETS
Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA
Project Period: 2000/08/01-2004/05/31

DESCRIPTION (provided by applicant): Tuberculosis is now second behind AIDS, as the World's most deadly microbial infection. However, a major fraction of AIDS patients die of mycobacterial infections, including TB. The TB problem is aggravated by the growing prevalence of drug-resistant TB, and especially multi-drug resistant (MDR) TB which cannot be treated with the front-line antibiotics for Mycobacterium tuberculosis. It is therefore important that targets be identified for development of new drugs for treatment of MDR TB. Suitable target enzymes should have biochemical functions essential for mycobacteria but with no similar function in mammals making it likely that drugs can be developed that will not lead to adverse reactions in humans. They should have well-defined assays suitable for screening of potential drugs. The proposed research elucidates the biochemistry associated with the production and utilization of the antioxidant thiol known as mycothiol. Mycothiol is produced only by mycobacteria, and other actinomycetes, and is not found in animals. The key genes for mycothiol biosynthesis have recently been identified and provide important potential new drug targets. Studies of MSH-deficient mutants indicate that mycothiol metabolism is involved in protecting against oxidative damage and in the detoxification of antibiotics, including one first-line TB drug. Although not essential for the laboratory culture of the model organism Mycobacterium smegmatis, current evidence suggests that mycothiol may be required for survival of M. tuberculosis in an oxygen rich environment. The present studies will determine the extent to which mycothiol is essential for survival of M. tuberculosis, will define the biochemistry involved in the first key step of mycothiol biosynthesis, and will determine how the biosynthesis of mycothiol is regulated. Methods used include new analytical and enzyme assays developed in these laboratories as well as established protocols in biochemistry and molecular biology. The results obtained will provide a key test of the suitability of mycothiol biosynthesis as a target for new TB drugs and will elaborate the biochemistry of a novel class of thiol important to a broad class of soil microorganisms, including most antibiotic producing bacteria.

Grant: 1R21AI051417-01A1
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: HSIA, RU-CHING PHD
Title: Polymorphic membrane proteins of *Chlamydia trachomatis*
Institution: UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD
SCHOOL
Project Period: 2003/05/01-2005/04/30

DESCRIPTION (provided by applicant): Chlamydial polymorphic membrane proteins (Pmps) are a newly identified family of *Chlamydia*-specific membrane proteins, whose role in chlamydial biology and pathogenesis is unknown. Genomic analysis of the prop family of *C. pneumoniae* has revealed frameshift mutations, deletions and gene duplications. Studies of the larger pmp families of *C. pneumoniae* and *C. psittaci* have also revealed that Pmp proteins are expressed in vitro, that some can be detected at the elementary body surface, and that some are dominant antigens during infection and may be targets for vaccine design. The emerging evidence is consistent with a role of the prop gene family in pathogenesis and immune evasion. The purpose of this project is to characterize the smallest pmp gene family identified to date: the 9-member family of *C. trachomatis*. In preliminary studies using the 9 partially purified recombinant Pmps as target antigens, I have observed differential Pmp-specific antibody responses in archived sera from patients with pelvic inflammatory disease. This analysis will be expanded through cross-sectional and longitudinal comparisons of Pmp-specific responses in a well-characterized patient population with genital *C. trachomatis* infection. This analysis may identify direct relationships between Pmp-specific responses and disease outcome. A second focus of this project will be to identify and characterize genetic and molecular determinants of Pmp expression in *C. trachomatis*. Polymorphisms will be identified and compared in the pmp families of selected study isolates. Experiments will be performed to characterize developmental patterns of pmp expression in these isolates. Using a panel of Pmp-specific monoclonal and polyclonal antibodies generated in this project, I will examine Pmp protein expression and eventual translocation to the surface of the outer membrane along development and at the single cell level using laser scanning confocal fluorescence microscopy.

Grant: 1R21AI052304-01A1
Program Director: CASSETTI, CRISTINA
Principal Investigator: LYLES, DOUGLAS S PHD BIOCHEMISTRY:FAT
AND LIPID
Title: Control of translation by VSV
Institution: WAKE FOREST UNIVERSITY HEALTH SCIENCES WISNTON-SALEM, NC
Project Period: 2003/09/29-2005/09/30

DESCRIPTION (provided by applicant): The control of translation plays a critical role in the pathogenesis of many viruses. The inhibition of host protein synthesis is important for suppression of the host antiviral response, while the selective translation of viral mRNAs is important for virus propagation in the host. Translational control by vesicular stomatitis virus (VSV), the prototype rhabdovirus, is one of the best-studied examples among the nonsegmented, negative-stranded RNA viruses. Previous experiments have made it clear that VSV uses novel mechanisms to control translation that are distinct from those of other well-studied RNA viruses, such as picornaviruses and influenza viruses. The goal of this project is to determine these mechanisms by which VSV inhibits host protein synthesis and promotes viral protein synthesis. Previous work has shown that the translation factor eIF2alpha is inactivated in VSV-infected cells as a result of phosphorylation by protein kinase R. However, we have shown that the cap-binding eIF4F complex is also inactivated in VSV-infected cells. Aim 1 is to determine how eIF4F is inactivated, and the relative contribution of eIF4F versus eIF2alpha inactivation in the inhibition of host protein synthesis in VSV-infected cells. Aim 2 is to determine the viral components responsible for inducing the inhibition of host protein synthesis. These studies will use new recombinant viruses to map the genes of VSV mutants that are either more effective or less effective than wild-type VSV in the inhibition of host translation. Aim 3 is to determine the basis of resistance of viral mRNAs to the inhibition of translation. These experiments will use chimeric mRNAs containing viral or host 5' and 3' untranslated regions to test for the presence of cis-acting sequences that enhance translation of viral mRNAs. We will also test recombinant viruses that express these chimeric mRNAs to determine whether mRNA synthesis by the viral transcriptase confers the resistance to the inhibition of translation. Upon completion of these experiments, we will have important new information about how viruses suppress the antiviral response in the host, and how they promote expression of their own gene products. The results of these experiments will be novel, because the mechanisms used by VSV differ substantially from those of other well-studied prototype viruses. Finally, it is very likely that there are host mRNAs that use mechanisms similar to those of VSV mRNAs to enhance their translation under conditions such as stress. Thus we expect to gain new insight into regulation of cellular translation under other conditions where the activities of translation initiation factors are inhibited.

Grant: 1R21AI053191-01
Program Director: RUBIN, FRAN A.
Principal Investigator: PAOLETTI, LAWRENCE C PHD
Title: Bacterial Vaccine Antigen Discovery
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 2003/09/30-2005/08/31

DESCRIPTION (provided by applicant): New directions in bacterial vaccine discovery may arise from studies of host-microbe interactions, particularly through the use of a newly described technology: the dynamic in vitro attachment and invasion system (DIVAS). DIVAS was developed to study bacterial attachment and invasion with cells held at specific and controlled conditions of growth, metabolism, and nutrient levels. Results from experiments performed with DIVAS and group B Streptococcus (GBS) type III strains substantiated earlier findings that capsular polysaccharide is not critical for invasion of respiratory epithelial cells. Moreover, GBS invaded these cells only when held at a fast as opposed to a relatively slower rate of growth and they expressed several proteins solely under growth conditions conducive for invasion. In this proposal, we seek to test the hypothesis that GBS proteins involved with invasion of eukaryotic cells are new and important targets of protective immunity. GBS is a major cause of neonatal sepsis and meningitis, and is increasingly prevalent among nonpregnant adults and the elderly with underlying illnesses. Preclinical and clinical trials have been successfully performed with protein conjugate vaccines prepared with polysaccharides from most of the nine currently known GBS serotypes. GBS protein antigens with virulence properties have been described and some with vaccine potential have been tested preclinically. In this proposal, we seek to use DIVAS to identify physiological conditions conducive for bacterial attachment/invasion of eukaryotic cells using GBS as a model pathogen. We plan to isolate and identify GBS membrane proteins expressed solely under invasive conditions (Specific Aim 1). Several of these newly expressed proteins will be purified directly from GBS, or cloned and recombinantly expressed, and tested as vaccine candidates in mice (Specific Aim 2). Findings from these studies utilizing this unique approach to vaccine antigen discovery could be directly applied to other bacterial pathogens including those on the category A bioterrorism list.

Grant: 1R21AI053270-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: VAN DER GOOT, FRANCOISE G PHD
Title: Anthrax toxin-host cell interactions
Institution: UNIVERSITY OF GENEVA GENEVA,
Project Period: 2003/09/01-2005/08/31

DESCRIPTION (provided by applicant): *Bacillus anthracis*, the causative agent of anthrax, produces two major virulence factors: the capsule and the anthrax toxin. Anthrax toxin is composed of three independent polypeptide moieties: the protective antigen (PA), the lethal factor (LF) and the edema factor (EF), which act in binary combinations. Anthrax toxin constitutes a typical A-B type toxin in the sense that LF and EF are endowed with enzymatic activities, the targets of which are cytoplasmic. Binding to the host cell and translocation of the enzymatic moieties into the cytoplasm are ensured by PA. Therefore, the role of PA is absolutely essential since neither LF nor EF exert any effect on mammalian cell in the absence of PA. Due to our long-standing interest and expertise in bacterial toxins and the recent bio-terrorist attack in the USA, we wish to contribute to a better understanding of the mode of action of the anthrax toxin. The aim of the present project is to study the interaction of PA with the target cell plasma membrane at the molecular level, to determine by what exact pathway the toxin is internalized by the cell and to identify the precise stage of the endocytic pathway where the enzymatic moieties must be translocated into the cytoplasm in order to reach their real target. To address these issues we will use morphological (microscopy), biochemical and cell biological approaches. Our combined expertise in cell biology, in bacterial toxins and in cellular microbiology will be a great asset in studying the anthrax-host cell interaction. These studies should not only increase our understanding of the mode of action of the anthrax toxin and thereby of anthrax pathogenesis but might also lead to the identification of potential therapeutic targets to treat the disease.

Grant: 1R21AI053347-01A1
Program Director: ZOU, LANLING
Principal Investigator: KISH, PHILLIP E PHD
Title: Conjugate Carriers for Delivery of Anthrax DNA Vaccine
Institution: TSRL, INC. ANN ARBOR, MI
Project Period: 2003/09/30-2005/09/29

DESCRIPTION (provided by applicant): Of the numerous biological agents that may be used as weapons, the Working Group on Civilian Biodefense has identified *Bacillus anthracis* as one of the most serious agents. The most effective defense against these agents on a broad scale is through an aggressive vaccination: program. Currently, there is one vaccine for anthrax approved for use in the United States, produced by the BioPort Corporation in Lansing Michigan. With recent events foreshadowing broader usage of vaccines against these biological agents in the general population, there has been substantial interest in the development of DNA-based vaccines due to the potential advantages associated with this approach. Genetic immunization, rather than direct delivery of recombinant proteins is beneficial since the potential for introduction of co-purifying contaminating proteins is eliminated. Although genetic vaccines typically have utilized naked plasmid DNA (pDNA) injections into muscle tissue to generate the immune response, the issue of vaccine stability and effectiveness will probably preclude their wide spread use. We are developing a novel technology for DNA delivery. The carrier has great utility for gene delivery, particularly in the area of genetic vaccines. In this application, we propose to examine the DNA binding and expression characteristics of the conjugate in vitro and in vivo, using a series of analogs and a mammalian expression plasmid that encodes potent anthrax antigens. The goal of this project will be the generation of information on how well carrier/pDNA complexes elicit an immune response in an animal model. These results will allow the evaluation of the effect of structural changes on the binding affinity for the pDNA and the resultant effect on immune response. To aid in the development of the technology for DNA vaccines, we have established collaborations with BioPort Corporation and Dr. Blake Roessler at the University of Michigan. We feel that development of this DNA delivery technology for either oral or injectable vaccines will have an immediate impact in the area of anthrax vaccine and will also have utility for a wide variety of other vaccines.

Grant: 1R21AI053359-01A1
Program Director: ZOU, LANLING
Principal Investigator: STEINHAUER, DAVID A BS
Title: Novel Vaccines for Anthrax Prevention
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 2003/07/15-2005/06/30

DESCRIPTION (provided by applicant): Anthrax is a highly lethal disease caused by *Bacillus anthracis*. Currently it poses a major concern as a bioterrorist weapon. The three-component toxin of *Bacillus anthracis* is the principle mediator of pathogenicity. It consists of the protective antigen (PA), the lethal factor (LF), and the edema factor (EF). Although antibody responses to the PA are known to protect anthrax, a suitable vaccine for prevention of inhalation anthrax, the most lethal form, is not currently available for large-scale practical use. We hypothesize that it might be possible to engineer particular domains of the PA into the hemagglutinin glycoprotein (HA) of influenza virus, and that vaccine strains of influenza containing such chimeric HAs might provide protective immunity against both diseases. One advantage is that influenza vaccines are produced in large scale on an annual basis, so mass immunization procedures would not be problematic provided the recombinant viruses grow reasonably well. Another advantage of this approach relates to the properties of influenza as a strong inducer of both systemic and mucosal immunity, and as such, might enhance the neutralizing antibody responses to PA and provide protection against inhalation anthrax. As such, we propose to generate chimeric HA-PA proteins, assess their capacity to provide HA receptor binding and membrane fusion functions, and generate viruses containing the most suitable constructs by reverse genetics. These viruses will then be analyzed in mice for their ability to induce appropriate anti-HA and anti-PA immune responses.

Grant: 1R21AI053394-01A1
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: PIERINI, LYNDA M PHD
Title: Screening for Inhibitors of F. tularensis Virulence
Institution: WEILL MEDICAL COLLEGE OF CORNELL NEW YORK, NY
UNIV
Project Period: 2003/07/15-2005/06/30

DESCRIPTION (provided by applicant): The gram-negative zoonotic bacteria, F tularensis is the causative agent of tularemia, a severe and sometimes fatal illness that can be caused by inoculation or inhalation of as few as 10 organisms. The extreme infectivity, ease of dissemination and capacity for disease and death, make the potential use of F tularensis as a biological weapon a serious concern. Despite this, there has been little basic science research on this pathogen. It is known that the macrophage is central for both the pathology and resolution of F tularensis infections. Accordingly, a molecular understanding of F tularensis interactions with macrophages is crucial if we hope to modify the course of disease with therapeutics. Chemical genetics provides a way to rapidly advance both fundamental and applied research on this genetically intractable microbe and we propose to use this approach, coupled with high resolution quantitative fluorescence microscopy to address the following two questions: First, how does F tularensis infect host cells? There is conflicting evidence regarding the entry mechanism used by this organism. It remains to be determined if F tularensis invades cells by phagocytosis, endocytosis or an active invasion mechanism. We will use fluorescence microscopy and pharmacological and molecular inhibitors to investigate the entry mechanisms, and we will use automated fluorescence imaging to screen a small molecule library for compounds that block macrophage infection. Knowledge of viral entry mechanisms is currently providing the basis for development of new therapeutics for HIV, so analogous information may be similarly helpful for protecting against F tularensis. Second, what are the properties of the intracellular niche occupied by F tularensis? Vacuoles inhabited by Mycobacteria, for example, are slightly acidic and this fact is being exploited for targeted delivery of antibiotics. There is scant data about the nature of the F tularensis vacuole. We will measure the pH of F tularensis-containing vacuoles using ratiometric fluorescence imaging, determine if vacuoles can fuse with early endosomes and/or lysosomes, and characterize the protein constituents of the vacuole. We will then use a fluorescence imaging-based screen to identify cell-permeable molecules that alter the intracellular fate of F tularensis. Small molecule modulators of F tularensis-macrophage interactions will be used in future experiments to identify virulence factors, and may provide leads for post-attack prophylaxes.

Grant: 1R21AI053411-01A1
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: TELFORD, SAM R DSC
Title: Proximal determinants of risk for tularemia outbreaks
Institution: TUFTS UNIVERSITY BOSTON BOSTON, MA
Project Period: 2003/09/30-2005/08/31

DESCRIPTION (provided by the applicant): Although much is known about the biology of *Francisella tularensis*, the factors that regulate its natural prevalence remain un-described. We seek to test the 'rule of the incumbent' hypothesis: by analogy with politics, *F. tularensis* epizootics in the northeastern United States are limited by interactions with a 'ruling' endosymbiotic *Francisella* sp. (previously classified as *Wolbachia* sp.) common within tissues of dog ticks, *Dermacentor variabilis*. The recent outbreak of tularemia on Martha's Vineyard provides a unique opportunity for identifying factors that serve as the basis for increased transmission of this rare infection because sites there appear to be longstanding natural foci of this infection. However, testing this hypothesis requires identification of these natural foci, which may comprise small patches of vegetation; transmission is not homogeneously distributed over the entire 100,000-hectare island. Accordingly, we first seek to identify such natural foci by (1) determining whether striped skunks (*Mephitis mephitis*) serve as effective sentinels for *F. tularensis* transmission; and (2) complementing skunk-based predictions by enlisting landscapers and others who are occupationally exposed to tularemia and other tick borne infections to actively report, in real time, the presence of animal carcasses encountered during their work. Natural foci will be definitively confirmed by intensive longitudinal epizootiologic analyses (prevalence of *F. tularensis* infection in ticks and other potential vector arthropods; evidence of exposure in rodents and other animals). In this manner, we shall accumulate reliable study sites and the preliminary data required for a comprehensive test of the 'rule of the incumbent' hypothesis. In addition, our mapping of risky sites may serve as the basis for local public health measures. We anticipate that the epidemiologic and epizootiologic methods developed or validated during the proposed work may contribute towards enhanced investigations of tularemia outbreaks; new prevention strategies for those at risk of natural or illicit tularemia exposure; enhanced detection of *F. tularensis* in the environment; and, ultimately, a better understanding of *F. tularensis*-host interactions.

Grant: 1R21AI053442-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: RYAN, EDWARD T
Title: Application of IVIAT to Bacillus anthracis
Institution: MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA
Project Period: 2003/08/01-2005/07/31

DESCRIPTION (provided by applicant): Bacillus anthracis is the cause of cutaneous, inhalation, and gastrointestinal anthrax. B. anthracis has also recently been used as an agent of bioterrorism. The identification of bacterial genes expressed uniquely in vivo during infection with B. anthracis would improve our understanding of the molecular bacterial events that occur during anthrax, and could lead to improved therapeutics and (less likely) an improved anthrax vaccine. One recently developed technique that permits identification of bacterial genes expressed uniquely in vivo is IVIAT (in vivo induced antigen technology). In this procedure, convalescent serum collected from humans or animals infected with a pathogen of interest is absorbed against bacteria grown in vitro. Absorbed serum is then used to probe a genomic DNA expression library of the pathogen of interest in an E. coli host system. Reactive clones express an antigen expressed uniquely in vivo, and reactive genes and their products can be further identified and analyzed. Our hypothesis is that B. anthracis contains genes that are expressed uniquely in vivo during anthrax, and that identification of such genes and their products will lead to improved understanding of the pathogenic events that occur during anthrax. Our specific aim, therefore, is to apply IVIAT to B. anthracis using two sets of already collected convalescent sera: one from rhesus macaques that survived inhalational challenge with virulent Ames strain B. anthracis and cleared documented B. anthracis bacteremia as part of a fully approved anthrax study at the Centers for Disease Control and Prevention; the other from humans surviving naturally acquired cutaneous anthrax (collected as part of a fully approved collaborative study between Kazakhstani and CDC researchers). We designed our study to take into account the high lethality of anthrax and the presence of a B. anthracis capsule, and in our project, we will specifically not evaluate well characterized virulence factors of B. anthracis (such as exotoxin and capsule), but will focus our efforts on identifying previously unrecognized genes uniquely expressed in vivo. IVIAT is an established protocol in our laboratory, and this preliminary collaborative study should lay a foundation for subsequent analysis of identified B. anthracis genes and their products.

Grant: 1R21AI053506-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: KANE, RAVI S PHD
Title: The Design of Inhibitors of Anthrax Toxin
Institution: RENSSELAER POLYTECHNIC INSTITUTE TROY, NY
Project Period: 2003/08/01-2005/07/31

DESCRIPTION (provided by applicant): Anthrax is caused by the spore-forming bacterium *Bacillus anthracis*. The ability to deliver the spores in an aerosol and the high mortality rate of inhalational anthrax have led to the use of the spores as a biological weapon. Antibiotic treatment of inhalational anthrax can be ineffective late in the infection because high levels of toxin in the blood cause death. Our research proposal is focused on the development of inhibitors of anthrax toxin, a combination of three proteins secreted by the bacterium. Protective antigen (PA) binds a receptor and is cleaved by a protease, allowing the heptamerization of the cell-associated PA63 fragment. Heptamerization allows binding of the enzymatic toxin components, edema factor (EF) and lethal factor (LF), and triggers endocytosis of these complexes. The acidic environment of the endosome leads to the translocation of the enzymatic proteins to the cytosol where they exert their toxic effects. EF is an adenylate cyclase that impairs the innate immune response by a variety of mechanisms. LF is a protease that causes lysis of macrophages, which results in shock-like symptoms and death. The recent identification of the anthrax toxin receptor (ATR) will facilitate the development of molecules that inhibit anthrax toxin action. We will use two approaches to isolate inhibitors of the PA-ATR interaction and test these inhibitors for the ability to block the intoxication process in vitro. In the first approach, we will select a peptide that binds ATR and then attach multiple copies of this peptide to a polymeric backbone. The resulting polyvalent compound is predicted to bind ATR with higher affinity than the peptide alone and prevent binding of PA to cells. The second approach is based on our previous observation that a soluble fragment of ATR can protect cells from toxin in vitro. We hypothesize that a mutant fragment that binds PA with higher than wild-type affinity will be a more effective inhibitor. We will isolate this mutant through sequential rounds of random mutagenesis, selection, and recombination. These compounds may extend the time during which a case of anthrax can be treated successfully.

Grant: 1R21AI053548-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: ELLINGTON, ANDREW D PHD NUCLEIC ACID
BIOCHEMISTRY
Title: Auto Selection of aptamers binding to pXO1 proteome
Institution: UNIVERSITY OF TEXAS AUSTIN AUSTIN, TX
Project Period: 2003/08/01-2005/07/31

DESCRIPTION (provided by investigator): As recent events have demonstrated, *Bacillus anthracis* can and has been used as a weapon of bioterrorism. The detection, tracking, and interdiction of various *Bacillus anthracis* strains is therefore of great concern, as is the treatment of anthrax infection, in order to generate molecular tools that can serve as resources in all of these venues, we propose to select nucleic acid binding species (aptamers) against the proteomes of one of the virulence plasmids associated with *Bacillus anthracis*, pXO1. These experiments will serve as a starting point for the immediate development of biosensors capable of identifying *Bacillus anthracis*, and will set the stage for future efforts in tracking, interdiction, and therapy. In particular, we plan to: 1. Increase the throughput of automated selection experiments. 2. Select aptamers against the pXO1 proteome. 3. Develop informatics methods for designing signaling aptamers. Overall, the significance of the proposed work can be succinctly summarized as follows: there are, no real-time or continuous methods for the detection of *B. anthracis*, primarily because there are no biopolymer reagents that can report molecular interactions without the need of immobilization or other processing steps (e.g., PCR, ELISA). The signaling aptamers we propose to develop will be unique in this respect. In addition, aptamers have a therapeutic potential that rivals that of monoclonal antibody or other protein drugs (see, for example, B.3). Therefore, we believe that the reagents developed during the execution of this application will be of great use to the entire biodefense community, and that investment in this work will have a significant multiplier effect. As a single example, based on our previous interactions with the military, we are poised to send any aptamers that are produced directly to the Critical Reagents Program of the Joint Program Office for Biologicat Defense (JPOBD) for further assay and adaptation to military sensor systems.

Grant: 1R21AI053554-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: ZHOU, DAOGUO PHD
Title: Germination of Bacillus anthracis Spores in Macrophages
Institution: PURDUE UNIVERSITY WEST LAFAYETTE WEST LAFAYETTE, IN
Project Period: 2003/07/01-2005/06/30

DESCRIPTION (provided by applicant): Anthrax is caused by the inhalation, ingestion or uptake through a cut or abrasion of spores of Bacillus anthracis. An essential step in B. anthracis pathogenesis is the transformation of metabolically dormant spores into vegetative, toxin-producing bacteria. Past research has primarily focused on the mechanism of macrophage killing by anthrax toxins. Recent studies have begun to explore factors that are responsible for the germination of B. anthracis spores and their subsequent survival in the macrophages. The spores are engulfed by macrophages where they germinate and commence the synthesis of toxins. Subsequent release from macrophages leads to proliferation in the blood of vegetative bacteria and extensive toxin production. Spore germination within the macrophage is a critical but not well-understood step in the infection process. It is not currently known which vacuolar compartment or spore properties are essential for germination. Our working hypothesis is that germination requires a specific endosomal/phagosomal compartment with appropriate physical and nutritional factors recognized by engulfed spores from pathogenic isolates and that germination requires specific receptors and/or specific spore surface properties. In order to test this hypothesis, we propose two approaches: 1) initially direct or limit spores to different phagosomal compartments and then block vacuole rearrangement at distinct steps in order to examine the effects on spore uptake and germination. 2) Select for macrophage-specific, germination deficient spores and thus identify spore components essential for intracellular germination. Results from our study will define the compartment of the vacuoles required for B. anthracis germination, and the macrophage-specific germination-deficient B. anthracis mutants should help to elucidate spore components essential for germination in macrophages. These results will advance our understanding of in vivo spore germination and the properties of B. anthracis spores necessary for this process. This information could be helpful for developing pharmaceutical agents useful for preventing the germination of B. anthracis spores inside macrophages.

Grant: 1R21AI053578-01A1
Program Director: ZOU, LANLING
Principal Investigator: FREYTAG, LUCIA C PHD
Title: NOVEL IMMUNIZATION STRATEGIES AGAINST ANTHRAX
Institution: TULANE UNIVERSITY OF LOUISIANA NEW ORLEANS, LA
Project Period: 2003/08/15-2005/07/31

DESCRIPTION (provided by applicant): Anthrax is caused by the Gram-positive bacterium *Bacillus anthracis*. Disease in humans results from contact with infected animals, contaminated animal products or after exposure to accidentally or intentionally released spores of *B. anthracis*. The distribution of anthrax spores through the US mail has underscored the sense of urgency for the development of improved vaccines against this bacterial pathogen. The current human U.S. anthrax vaccine consists of aluminum hydroxide-adsorbed culture supernatant from a non-encapsulated strain of *B. anthracis*. This vaccine requires up to six intramuscular vaccine doses given over 18 months. In addition to Protective Antigen, the vaccine also contains a number of other bacterial and media-derived proteins which are likely responsible for the adverse effects experienced by some individuals and may reduce the efficacy of this vaccine. An ideal vaccine against anthrax should be safe, easy to deliver, provide long-lasting protection, require only one or a few doses, and be effective against different strains of *B. anthracis*. Recent advances in vaccine development have demonstrated that mucosal and transcutaneous immunization in the context of an appropriate adjuvant can produce both humoral and cellular antigen-specific immune responses in both the mucosal and systemic compartments of the host. Such needle-free immunizations are easy to deliver, cost-effective, and suitable for mass immunization campaigns, such as would be necessary during a national emergency or for vaccines delivered to underdeveloped countries. In this application, we will test the hypothesis that mucosal or transcutaneous vaccination with anthrax antigens can induce strong, sustained, and effective immune responses associated with protection against infection or can prime effectively for the induction of these responses. Challenge studies will allow us to correlate immune responses with protective efficacy and determine the contribution of mucosal immune responses to protection against pulmonary anthrax. The results obtained from these studies will contribute to our understanding of the host responses involved in protection against anthrax and to the formulation of improved anthrax vaccines. This information may also be valuable in the development of similar prevention strategies against other potential agents of biowarfare/bioterrorism.

Grant: 1R21AI053607-01A1
Program Director: KLEIN, DAVID L
Principal Investigator: GREENSPAN, NEIL S MD PATHOLOGY:CLINICA
Title: Pathogen and Host Proteins in Pneumococcal Immunity
Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH
Project Period: 2003/09/29-2005/09/30

DESCRIPTION (provided by applicant): Streptococcus pneumoniae (pneumococcus) is a major worldwide pathogen of humans, causing serious infections in the lungs, central nervous system, blood, and middle ear. Current vaccines and antibiotics do not provide equal or adequate protection for individuals of all ages, infections at all sites, or against pneumococci of all strains (serotypes). Therefore, it would be valuable to devise a pneumococcal vaccine based on relatively invariant pathogen-encoded proteins that are present in all or most strains and that can elicit protective antibodies. In addition, it would be valuable to be able to augment the abilities of these proteins to elicit protective antibody responses. Aim 1. To determine if the host-encoded immune system molecules, BLyS and C3d, can enhance potentially protective immune responses to the pneumococcal protein PspA. Studies under this aim will also address the mechanisms by which host proteins mediate any enhancements of the immune responses to PspA. Aim 2. To determine if a protein derived from the pneumococcus (PspA) can be more effective than a non-pneumococcal protein, when physically linked to pneumococcal capsular polysaccharides (PS), at enhancing the PS-specific serum antibody response. Aim 3. To determine how, in comparison to normal mice, mice lacking the taci gene (involved in anti-PS antibody responses) respond to pneumococcal PS-protein conjugate vaccines and to pneumococci. This aim will also determine if taci-deficient mice, immunized or unimmunized, have increased susceptibility to pneumococcal infection.

Grant: 1R21AI053842-01A1
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: GOLDBERG, JOANNA B.
Title: Pseudomonas aeruginosa LPS: A Post-Genomic Analysis
Institution: UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA
CHARLOTTESVILLE
Project Period: 2003/07/15-2005/06/30

DESCRIPTION (provided by the applicant): The long-term goal of this research is to understand the role of lipopolysaccharide (LPS) in the virulence of *Pseudomonas aeruginosa* in order to develop novel therapeutics and vaccines to combat this important pathogen. The O antigen portion of *P. aeruginosa* LPS is required for virulence in acute infections and its loss is critical for chronic lung infections in cystic fibrosis patients. The experiments in the parent grant are directed at defining the pathway of synthesis of the O antigen from the serogroup O11 strain PA103, determining the transcriptional organization of this O antigen locus, and testing O antigen mutants in two different mouse models of infection. The goal of this exploratory grant is to understand how *P. aeruginosa* O antigen is regulated. One of the conditions that has been shown to dramatically effect the expression of *P. aeruginosa* O antigen is temperature; at 45 degrees C, no O antigen is expressed. We will analyze RNA and protein products to determine whether O antigen is transcriptionally or post-transcriptionally regulated. Using genetic approaches, we will identify the O antigen regulator. We will mutate this temperature regulator and use post-genomic techniques including micro-array and proteomic analyses to identify additional genes and proteins that are regulated with O antigen. Uncovering the nature of this regulation will allow us to propose mechanisms to inhibit O antigen expression and thereby decrease the virulence of this bacterium in acute infections. Results obtained through this grant, in combination with the mutational analysis and virulence studies described in the parent proposal, may be beneficial in the development of new strategies that target this important opportunistic pathogen.

Grant: 1R21AI054395-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: ZHANG, HONG-ZHONG PHD
Title: Beta-lactamase Antibiotic Resistance of B anthracis
Institution: UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA
Project Period: 2003/05/01-2005/04/30

DESCRIPTION (provided by applicant): Recent anthrax-related bioterrorism incidents highlight the need for effective antimicrobial agents for treatment and prevention. Penicillin antibiotics has been historically the first choice for this purpose. However, concerns for drug resistance have resulted in recommendations that these drugs no longer be considered as drug of choice. This application proposes to define the biochemical and molecular basis of resistance to beta-lactams, as well as the potential for resistance to emerge in B. anthracis. The aims of the proposed experiments are: 1) to determine whether one or both of the two putative B-lactamase genes code for a B-lactamase and 2) to identify regulatory genes controlling B lactamase gene expression. Specifically, individual Beta-lactamase gene will be cloned and expressed. Encoded protein will be purified and in vitro biochemical studies will be performed to determine its substrate profile and inhibitor specificity. Candidate regulatory genes as identified by genome scanning will be cloned and their sequence will be determined. Mutants of regulatory genes will be constructed using genetic manipulation to assess the function of each gene product. The result of this study will elucidate the molecular mechanism underlying the resistance of Bacillus anthracis to penicillin antibiotics and inform decisions about the clinical utility of Beta-lactam antibiotics.

Grant: 1R21AI054468-01
Program Director: SCHMITT, CLARE K.
Principal Investigator: SPERANDIO, VANESSA PHD
Title: EHEC interactions with the normal intestinal flora
Institution: UNIVERSITY OF TEXAS SW MED DALLAS, TX
CTR/DALLAS
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): Enterohemorrhagic E. coli (EHEC) O157:H7 is responsible for major outbreaks of bloody diarrhea and hemolytic uremic syndrome (HUS) throughout the world. One of the major problems in the control and prevention of EHEC outbreaks is the fact that it has a very low infectious dose. EHEC colonizes the large intestine where it causes attaching and effacing (AE) lesions, which are believed to be the first step toward infection of the host, and also produces Shiga toxins (Stx), which are responsible for the major symptoms of HUS. The genes involved in the formation of these AE lesions are encoded within a chromosomal pathogenicity island named the Locus of Enterocyte Effacement (LEE). We recently reported that both the LEE and the genes encoding Stx are activated by a bacterial cell-to-cell signaling mechanism known as quorum sensing (QS). Bacteria secrete hormone-like compounds, called autoinducers, which interact with bacterial transcriptional regulators to drive gene expression. The QS mechanism employed in this activation is involved in bacterial inter-species communication, and we propose that activation of EHEC virulence genes by this system may occur in response to autoinducers produced by the normal intestinal flora. This could, in part explain the low infectious dose of EHEC. This grant application in response to RFA (AI-02-008) "Impact of microbial interactions on infectious diseases" intends to study EHEC virulence gene expression in response to signals produced by the normal intestinal flora. Given that this RFA (AI-02-008) is designed to investigate the impact of microbial interactions on infectious diseases, including the interactions between pathogens and the normal flora, we feel that this grant application is particularly well suited to the mission of the RFA. In Specific Aim 1, we propose to study EHEC gene expression at the genome level using DNA microarrays to assess EHEC responses to signals produced by the normal intestinal flora. In Specific Aim 2, we propose to monitor EHEC virulence gene expression in a mixed population in the presence of the intestinal flora.

Grant: 1R21AI054490-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: RUSSELL, DAVID G PHD PARASITOLOGY, OTH
Title: Metabolism of M. tuberculosis: New Targets for MDR TB
Institution: CORNELL UNIVERSITY ITHACA ITHACA, NY
Project Period: 2003/04/15-2005/03/31

DESCRIPTION (provided by applicant): Chemotherapeutic treatment of tuberculosis is a prolonged process due to the innate resistance of the bacterium to the drugs available, their relatively low tissue concentration and the limited number of targets. These parameters lead with alarming frequency to the development of single and multi-drug resistant strains. Initiatives towards identification of new targets are needed badly and this current proposal describes an integrated program to facilitate a more global analysis of metabolic pathways active under conditions relevant for infection. The experiments detail microarray analysis of bacterial RNA, coupled with a novel mutant screen to identify regulator elements responsible for mobilizing metabolic networks. The specific aims are: 1) Cataloging bacterial metabolism under specific growth conditions: Bacteria will be cultivated in a fermentor to generate a homogeneous population grown under defined, tightly-regulated, physiological conditions relevant to the intracellular environment (varying O₂ tension, pH, carbon source & element concentration). Patterns of gene expression will be documented by microarray analysis to identify genes up-regulated under relevant experimental conditions. 2) The intracellular validation of potential targets: The role of "target" genes will be probed through analysis of (i) intracellular expression profiles using green fluorescent protein (GFP) reporter constructs and real-time PCR and (ii) deletion of the gene and analysis of the growth characteristics in culture, and in macrophages. 3) Screening for sensor/regulator cascades operational under specific growth conditions: Once "target" genes have been identified we will identify the sensor/regulator pathways active in regulation of gene function using the GFP reporter constructs transfected into transposon-mutagenized bacteria and select for aberrant expressors using a FACScaliber sorter.

Grant: 1R21AI054595-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: SMILEY, STEPHEN T BS
Title: Priming CD4+ T cells to protect against pneumonic plague
Institution: TRUDEAU INSTITUTE, INC. SARANAC LAKE, NY
Project Period: 2003/03/01-2005/02/28

DESCRIPTION (provided by applicant): The etiologic agent of pneumonic plague is the facultative intracellular bacterium *Yersinia pestis*. As antibiotic-resistant strains of *Y. pestis* are known to exist and sophisticated bioterrorist attacks are plausible, there is an urgent need to develop pneumonic plague vaccines. Subunit vaccines comprised of the *Y. pestis* F1 and/or V proteins provide experimental animals with significant protection against pneumonic plague. Further development of these vaccines will undoubtedly be aided by a better understanding of how vaccination protects against plague. While it is clear that antibodies contribute to the protective response, CD4 + T cells must also participate, as antigen-specific CD4 + T cells are critically important for memory B cell responses and the affinity maturation of antibodies. Relevant prior studies have been limited in scope, although they established that the F1 and V proteins do elicit significant CD4 + cell responses. In addition to stimulating, maintaining and/or boosting antibody responses, vaccine-elicited CD4 + T cells could also direct cellular immunity at host cells harboring *E. pestis* organisms, and/or pathologically contribute to vaccine-related adverse reactions. Thus, detailed studies of *Y. pestis* vaccine elicited CD4 + T cells are clearly warranted. Prior to defining the functional capacities of vaccine-elicited CD4 + T cells, one must first develop appropriate model systems. In Aim 1, we will determine the immunodominant epitopes recognized by V protein-specific CD4 + T cells and develop assays for enumerating V-specific T cells. In Aim 2, we will use those assays to define vaccination conditions that optimally prime effector and memory V-specific CD4 + T cells. We will also define conditions that recruit V-specific CD4 + T cells to pulmonary tissues, which are the primary target of pneumonic plague. In Aim 3, we will determine whether the same vaccination conditions optimally prime both B and T cell responses. Those studies may immediately suggest means to improve vaccine efficacy and/or limit adverse reactions. The primary goals of this R21 proposal are to develop assays and vaccination protocols that will enable future studies to measure their capacity of *E. pestis*-specific CD4 + T cells to protect against pneumonic plague, and to dissect the relative importance of vaccine-elicited B and T cell responses. Our long-term goals are to use that information to develop effective pneumonic plague vaccines.

Grant: 1R21AI054602-01
Program Director: BEANAN, MAUREEN J.
Principal Investigator: MATSUMURA, ICHIRO BS
Title: Engineered alkaline phosphatases as biosensors
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): *Bacillus anthracis* spores are currently detected by established but slow microbiological test procedures. The development of faster, cheaper and higher-throughput detection methods would enable more effective responses to bio-terrorism and natural infectious disease outbreaks. Our goal is to engineer a reporter enzyme so that is inactive until it encounters a pathogen marker. Our design strategy is to imitate the twostep natural evolution of "intrasteric" regulation. We have already generated a variant of the *Escherichia coli* beta-galactosidase (BGAL) that is specifically activated 5.7-fold when co-expressed with the human immuno-deficiency (HIV) protease. We believe that the *E. coli* alkaline phosphatase (AP) has even greater potential as a biosensor, and propose studies with the following specific aims: 1. to isolate effector-dependent AP variants with greater response to the HIV protease (>570% activation) and more robust enzyme activities. 2. to "re-program" the best biosensor so that its activity becomes dependent upon the *B. anthracis* Lethal Factor (LF), the anti-influenza hemagglutinin (HA) antibody, or the anti-*Yersinia pestis* F1 antibody. 3. to array biosensors that respond to different effectors upon a chip for the rapid detection of pathogen markers. The biosensors generated in this study will streamline disease diagnosis by supplanting time-consuming and expensive immunoassays. These experiments will test the feasibility of our evolutionary hypothesis and demonstrate the utility of novel protein engineering techniques.

Grant: 1R21AI054614-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: GILMORE, MICHAEL S AB
Title: Resistant Enterococci in the GI tract Consortium
Institution: UNIVERSITY OF OKLAHOMA HLTH SCIENCES CTR OKLAHOMA CITY, OK
Project Period: 2003/02/15-2005/01/31

DESCRIPTION (provided by applicant): Enterococci are leading causes of nosocomial infection. Accumulating evidence indicates that multiple antibiotic resistant Enterococcal strains in the hospital environment colonize the GI tracts of patients within hours or days of admission. From the patient's own GI tract, the organisms are well positioned to colonize surgical sites, catheterized bladders, or invade the bloodstream from breaches in the integrity of the colon. Currently, little is known about the allogenic or autogenic factors that lead to GI tract colonization by these multiple resistant strains, and it is not known whether this colonization is competitive or non-competitive with indigenous commensal Enterococcal strains. We recently characterized a pathogenicity island that encodes precisely the types of traits that would be expected to alter the Enterococcal colonization pattern, including PTS uptake systems for metabolism of new carbohydrates, new surface adhesions, and a bile acid hydrolase that may render previously uninhabitable locations within the GI tract amenable to colonization by pathogenic Enterococcal lineages. The main barriers to progress in this area are the complexity of the GI tract flora compounded by patient-to-patient variability. To begin examining the first principles that dictate relationships between Enterococci and other organisms in the GI tract consortium, the occurrence of Enterococci within a simple in vitro model of the human GI tract consortium will be characterized. The GI tract consortium model will be established, and it will be determined whether *Enterococcus faecalis* and *E. faecium* exhibit colonization preferences for particular niches within the model, and whether they home to the same niche. It will further be determined whether commensal and pathogenic lineages of *E. faecalis* and *E. faecium* partition to the same location within the model. Organisms found to share the same niche as clinical isolates, and potentially contributing to a symbiotic relationship will be identified and tested, as will organisms occurring in sites from which Enterococci are excluded. Finally, the effect of antibiotics in shifting the colonization pattern of resistant clinical isolates in this in vitro model will be characterized. Based on these results, well-grounded specific questions can be postulated and tested subsequently to verify their importance in vivo, ultimately leading to the design of agents to prevent colonization by antibiotic resistant strains.

Grant: 1R21AI054630-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: FIKRIG, EROL MD MEDICINE
Title: CO-INFECTION WITH B BURGENDORFERI AND A PHAGOCYTOPHILA
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 2003/02/01-2005/01/31

DESCRIPTION (provided by applicant): Lyme disease and human granulocytic ehrlichiosis (HGE), caused by *Borrelia burgdorferi* and *Anaplasma phagocytophila* respectively, are two common vector-borne illnesses in the United States. Both pathogens are transmitted to man by *Ixodes scapularis* ticks, and dual infections have been documented in the arthropod and vertebrate hosts. The purpose of this application is to determine whether co-infection of mice with *B. burgdorferi* and *A. phagocytophila* alters *B. burgdorferi* infectivity and the severity of murine Lyme arthritis. Our recently published report showed that the simultaneous experimental infection of mice with *B. burgdorferi* and *A. phagocytophila* increased spirochete numbers and the severity of joint inflammation. We will now explore this further by determining how the challenge dose of each organism and the timing of exposure of each pathogen influences the course of murine Lyme borreliosis. We will also determine whether these differences are observed when *B. burgdorferi* and *A. phagocytophila* are transmitted via tick bite rather than needle inoculation. Our published studies have also demonstrated that antibodies against *B. burgdorferi* genes that are expressed *in vivo* contribute to immunity against the spirochete and that *B. burgdorferi* gene expression can be influenced by the host immune response. We will now use spirochete DNA microarrays that we have developed to explore the hypothesis that *B. burgdorferi* gene expression *in vivo* is modified during co-infection with *A. phagocytophila*. We will identify *B. burgdorferi* genes that have altered levels of expression during co-infection and examine the role of these gene products in immunity against infection and the severity of joint inflammation. These studies will more clearly define the influence of *B. burgdorferi* and *A. phagocytophila* co-infection on the course of murine Lyme arthritis and explore the mechanisms by which dual infection alters spirochete infection. These efforts should increase our understanding of the importance of co-infection with these tick-borne agents on the outcome of disease and serve as a general model for how dual infection can influence host responses and pathogen gene expression.

Grant: 1R21AI054647-01
Program Director: KLEIN, DAVID L
Principal Investigator: WEISER, JEFFREY N
Title: Competition Among Bacterial Respiratory Pathogens
Institution: UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): Acute respiratory infection (ARI) is a major cause of morbidity and mortality. This application focuses on two of the most prominent causes of ARI, *Streptococcus pneumoniae* and non-typeable *Haemophilus influenzae* (NTHi). Colonization of their common niche, the nasopharynx, is extremely common for both species and represents the first step in their pathogenesis. Recent clinical data suggests that vaccination resulting in reduced carriage of one species may exacerbate disease caused by the other. This observation, together with preliminary data confirming that these species compete in vitro and in animal models of colonization, form the rationale for this application to determine the specific mechanisms of competition between *S. pneumoniae* and NTHi. The approach will focus on themes that are common in their pathogenesis. Both organisms express cell-surface phosphorylcholine (ChoP), an unusual bacterial structure that allows for binding to epithelial cells expressing the receptor for platelet activating factor (rPAF) but renders the organisms sensitive to innate immunity mediated by C-reactive protein (CRP). In addition, a neuraminidase expressed by *S. pneumoniae* can desialylate host receptors and the sialylated LPS of *H. influenzae*. Competition involving bacterial phosphorylcholine and the effects of neuraminidase will be analyzed in three highly exploratory specific aims. Aim 1 will test the hypothesis that there is direct bacterial-bacterial interaction that results in competition in their common niche, the mucosal surface of the nasopharynx. The effect of pneumococcal neuraminidase on NTHi in a chinchilla model of nasopharyngeal colonization and competition involving choline acquisition in vitro will be determined. Aim 2 examines whether bacterial interactions with respiratory tract epithelial cells promote competition in adherence involving binding via the rPAF or by desialylation of host receptors. Aim 3 explores whether the host's epithelial cell inflammatory response and the elaboration of TNF α and CRP favor adherence and/or colonization of one species over the other. The information obtained will facilitate a longer-term effort to understand the molecular mechanisms for competition between these important pathogens in their common niche. Future strategies to decrease disease burden by reducing carriage could result from a greater understanding of interactions between species.

Grant: 1R21AI054655-01
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: TAO, LIN PHD MEDICAL
MICROBIOLOGY & IMMUN
Title: Viral Infection in Lactobacilli: An Animal BV Model
Institution: UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL
Project Period: 2003/03/15-2005/02/28

This application is in response to the NIH RFA (AI-02-008) entitled "Impact of Microbial Interactions on Infectious Diseases." Specifically we will study the interaction between viruses (phages) and vaginal lactobacilli during the development of experimental bacterial vaginosis (BV) in animals. BV is the most common vaginal disorder affecting women worldwide. Because the cause is unknown, no methods are available to prevent BV. Although BV itself only has mild discomfort, such as discharge and fishy smell, BV is associated with two major health risks in women: preterm delivery and increased susceptibility to contract HIV. Both incidences kill millions of newborns and adults annually. Therefore, it is urgent to study the cause of BV, because discovering its cause will be a key step in developing more effective ways to prevent and cure the disease. In healthy women, lactobacilli dominate the vaginal microbial ecology. During BV, a shift in microbial dominance occurs-lactobacilli decrease while Gardnerella vaginalis and anaerobic bacteria increase. It is unknown, however, what triggers the shift in vaginal ecology to cause BV. We have isolated phages that infect vaginal lactobacilli. Because these phages can potentially shift vaginal microbial dominance, they are implicated as an underlying cause for BV. We hypothesize that BV may occur after phages infect vaginal lactobacilli. We will test this hypothesis according to Koch's postulates. Namely, a virus isolated from lactobacilli will be inoculated into an animal to cause BV in the animal. We will achieve two specific aims: 1) Study in vitro interactions between phages and monkey vaginal lactobacilli. 2) Establish a monkey BV model by shifting vaginal ecology with a Lactobacillus phage. Upon completion of the study, we expect to have developed a BV animal model based on Koch's postulates, documented that BV can be an infectious disease and that the infectious pathogen is the Lactobacillus phage. We will have an improved understanding of the BV etiology. This will be the first step in attaining our long-term goal: developing better methods to treat and prevent BV.

Grant: 1R21AI054673-01
Program Director: RUBIN, FRAN A.
Principal Investigator: CYWES, COLETTE PHD
Title: Synergistic infection by varicella and streptococci
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 2003/03/01-2005/02/28

DESCRIPTION (provided by applicant): Group A Streptococcus (GAS) invasive disease is characterized by the dissemination of bacteria from the skin surface or a superficial wound through the epithelial barrier, in a paracellular fashion, into underlying tissues. The incidence of invasive GAS disseminated infection is increased by approximately 50-fold in children with primary varicella zoster virus (VZV) infection (chickenpox). The association of GAS invasive infection with VZV and not with other skin-damaging disorders suggests that VZV infection results in specific changes in the skin that enhance susceptibility to GAS infection. The focus of this application is the hypothesis that VZV infection of the epidermis induces expression of the GAS receptor CD44, enhanced GAS binding to epithelial cells, and augments CD44-mediated cytoskeletal rearrangements leading to intercellular junction disruption. GAS binding to CD44 triggers a cell signaling cascade that results in disruption of cell-cell junctions, thereby facilitating the paracellular translocation of GAS and culminating in invasive disease. The specific aims of the application are: (1) to determine the effect of VZV on GAS association with keratinocytes; (2) to characterize the cytoskeletal changes induced by VZV infection of polarized keratinocytes and the effects on the GAS-CD44 mediated cytoskeletal activity; and (3) to define the effects of VZV infection on intercellular junction integrity, epithelial barrier function, and GAS translocation. By comparing uninfected, VZV-infected, and VZV- and GAS-co-infected polarized keratinocyte monolayers, these studies will systematically evaluate the specific cellular responses elicited by VZV and the mechanisms that impact on pathogenesis of invasive GAS infection. These results will advance our understanding of the intracellular cytoskeletal changes induced by VZV that lead to disruption of the epithelial barrier and may provide insight into the mechanisms of opportunistic co-infection by GAS. Ultimately results of this line of investigation may suggest strategies to limit bacterial complications of this and other viral infections.

Grant: 1R21AI054753-01
Program Director: CASSETTI, CRISTINA
Principal Investigator: ZHANG, JING-REN PHD
Title: Promotion of pneumococcal adherence by viral infection
Institution: ALBANY MEDICAL COLLEGE OF UNION ALBANY, NY
UNIV
Project Period: 2003/09/30-2005/09/29

DESCRIPTION (provided by applicant): Respiratory diseases caused by Streptococcus pneumoniae (pneumococcus) and influenza virus infections are major public health problems worldwide. Many studies have suggested a synergistic interaction between S. pneumoniae and influenza virus in causing human diseases. It is well known that influenza virus infection of the upper respiratory tract predisposes to secondary infections caused by S. pneumoniae in humans and experimental animals. In fact, most mortality during influenza epidemics arises from secondary bacterial infections including pneumococcal infection. Therefore, unraveling this synergy by therapeutic interventions is an attractive strategy to control human diseases caused by S. pneumoniae and influenza virus infections. Although the mechanisms of this virus-bacterial synergy remain to be defined, recent studies have implicated that viral infection may promote pneumococcal infection by promoting bacterial adhesion to respiratory epithelium. Our long-term goal is to understand how this virus-pneumococcus synergy can be modulated therapeutically for effective control of pneumococcal infection. The objective of this application is to determine how viral infection promotes pneumococcal adhesion to epithelial cells. We hypothesize that viral infection promotes pneumococcal adhesion by modulating the expression of cellular receptors. We will test our hypothesis and achieve the objective of this application by pursuing the following two specific aims. Specific Aim 1: We will determine host epithelial receptors for S. pneumoniae that are induced by influenza virus. Primary human respiratory epithelial cells will be infected with influenza virus to identify virus-induced epithelial membrane proteins by proteomic approach. The virus-induced proteins will be further evaluated for potential interactions with S. pneumoniae. Specific Aim 2: We will identify bacterial surface proteins that are involved in virus-promoted adhesion by signature-tagged mutagenesis. It is our expectation that these approaches will identify novel ligand-receptor interactions required for bacterial adhesion. These results will be significant because they are expected to open up new areas for future investigation of virus-bacterial interaction in microbial pathogenesis. Furthermore, novel pneumococcal proteins and host receptors identified in this application may provide new targets for preventive interventions.

Grant: 1R21AI054762-01
Program Director: SCHMITT, CLARE K.
Principal Investigator: WEISS, ALISON A
Title: Shiga Toxin Encoding Phage and Intestinal E.coli
Institution: UNIVERSITY OF CINCINNATI CINCINNATI, OH
Project Period: 2003/02/15-2005/01/31

DESCRIPTION (provided by applicant): Shiga toxin-producing Escherichia coli, including O157:H7, are emerging pathogens of major importance. Bacterial colonization is associated with intestinal disturbances; however, the severe, potentially fatal symptoms of bloody diarrhea (hemorrhagic colitis) and destruction of the kidney leading to hemolytic uremic syndrome (HUS) are due to a bacterial toxin, Shiga toxin. The genes for Shiga toxin are encoded by a temperate bacteriophage in the late gene operon. Shiga toxin is expressed when the phage lytic cycle is induced, along with the genes necessary for production of viral particles and bacterial lysis. Phage-mediated bacterial lysis is necessary for toxin secretion. The viral particles produced during infection with the pathogenic O157:H7 can infect the nonpathogenic intestinal E. coli. When this occurs, the previously harmless E. coli will produce Shiga toxin and amplify the pathogenic process. Our preliminary data suggest that the Shiga toxin production by intestinal flora can be substantial and that severe, possibly life-threatening disease is more likely to occur in individuals whose intestinal flora can be infected by the Shiga toxin phage. In contrast, individuals with intestinal flora that are resistant to the Shiga toxin phage will be protected from severe disease. Currently, it is difficult to prevent disease by E. coli O157:H7 since the infectious dose is very low, and antibiotic treatment, instead of being beneficial, can enhance progression to fatal disease. We propose to develop methods to convert the intestinal flora to phage-resistance as a therapeutic approach to controlling disease caused by Shiga toxin-producing pathogens. This is especially important since there is no treatment, other than supportive care, once disease has developed. The specific aims of this application are to: 1) Characterize Shiga toxin production by clinical isolates of O157:H7 in the presence of susceptible or resistant human intestinal E. coli; and 2) Develop methods to convert intestinal flora to phage resistance using a mouse model of disease.

Grant: 1R21AI054802-01
Program Director: CASSETTI, CRISTINA
Principal Investigator: MCCULLERS, JONATHAN A MD
Title: Influenza-pneumococcal synergism: role of neuraminidase
Institution: ST. JUDE CHILDREN'S RESEARCH HOSPITAL MEMPHIS, TN
Project Period: 2003/04/01-2005/03/30

DESCRIPTION (provided by applicant): A lethal synergism exists between influenza virus and *Streptococcus pneumoniae*, accounting for excess mortality during influenza epidemics. The recent development of a mouse model recapitulating lethal synergism allows study of potential mechanisms underlying this synergism. The hypothesis that the sialidase activities of the viral and bacterial neuraminidases (NAs) act synergistically to promote adherence and invasion of pneumococcus will be explored. Human viruses of different subtypes and viruses engineered by reverse genetics to have different total NA activities and relative specificities for alpha (2-3) and alpha (2-6) sialic acids will be studied in bacterial adherence assays and in mouse and ferret models of synergism. The concept that differences in excess mortality based on viral subtype can be related to differences in the activity and specificity of the viral NA will be studied by comparison of these results with historical excess mortality related to particular viruses. The utility of viral NA inhibitors for abrogation of synergism will be evaluated. A detailed understanding of the interaction between influenza virus and pneumococcus will provide support for drug and vaccine based interventions aimed at amelioration of human disease and death caused by pneumococcal superinfection following influenza. More broadly, the proposed work addresses evidence that treatment of viral diseases can change the subsequent outcome of bacterial infections. The research proposed is consistent with the purpose of the RFA as it involves an intermicrobial interaction well known to cause significant human mortality, and it makes use of novel technology (reverse genetics for influenza) and novel animal models.

Grant: 1R21AI054808-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: COLEMAN, JAMES L MS
Title: Gene expression of Borrelia during Babesia coinfection
Institution: CENTER FOR COMMUNITY HEALTH RENSSELAER, NY
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): Coinfection with *Borrelia burgdorferi* and *Babesia microti* is a clinically documented, emerging public health concern in the Northeastern United States. However, to date, it still remains unclear whether or not concurrent infection with these pathogens leads to greater disease severity in humans. To explore whether concurrent infection with these two emerging human pathogens leads to greater disease severity, we have proposed to develop an animal model to quantify the polymicrobial burden as well as several clinical parameters during single and concurrent experimental infection in mice of different predisposing conditions and genetic backgrounds. In addition to evaluating disease severity during single and concurrent *Borrelia* and *Babesia* infection in mice, we will also monitor the differential genetic expression of *B. burgdorferi* in response to *B. microti* in feeding larval ticks and infected mouse tissues utilizing whole genome DNA arrays. To further define the innate immunological response of the vertebrate host during polymicrobial infection, genomic expression analysis will be carried out in murine and human monocytic cells stimulated with live *B. burgdorferi* and *B. microti* alone and concurrently.

Grant: 1R21AI054831-01
Program Director: KORPELA, JUKKA K.
Principal Investigator: HULL, RICHARD A
Title: Bacterial interactions in biofilms
Institution: BAYLOR COLLEGE OF MEDICINE HOUSTON, TX
Project Period: 2003/02/01-2005/01/31

DESCRIPTION (provided by applicant): Urinary tract infection (UTI) is the most common hospital acquired infection and is usually associated with the use of an indwelling catheter. Bacteria colonize the bladder and form a slimy coat on the catheter called a biofilm. Bacteria in a biofilm are able to evade host defense mechanisms and also become less susceptible to antibiotic therapy. As a consequence, catheter associated UTI is more difficult to eradicate. The long-term goal of this project is to find new ways to interfere with bacterial biofilm formation so that treatment of catheter associated UTI is more effective. Most previous genetic studies of *Escherichia coli* bacteria in biofilms have focused on biofilms composed of only one type of bacteria. These 1-species biofilms usually contained the laboratory strain *E. coli* K-12. However biofilms in nature are typically composed of a consortium of organisms. The bacteria within the biofilm community may communicate among themselves. For example, they may share, or compete for, metabolic products or they may respond to extracellular signals. The outcome of these microbial interactions may be symbiotic or competitive. Certainly the microenvironment of a multi-species biofilm is different from a mono-culture biofilm that contains only *E. coli* K-12. We propose to study the gene expression of a clinical *E. coli* that is growing in a biofilm together with *Enterococcus faecalis*. Each of these bacterial species is a common cause of catheter associated UTI. A genomic approach will be used to discover genes that are key to formation of 2-species biofilms. High throughput genetic methods will be used to identify *E. coli* genes that are differentially expressed in a 2-species biofilm and genes that are uniquely required for formation of 2-species biofilms. Future studies will identify the mechanism of action of each of the genes discovered. Results of the proposed experiments may illuminate new targets for intervention therapies that disrupt biofilm formation. This may lead to more effective treatment of catheter-associated infections.

Grant: 1R21AI054834-01
Program Director: CASSETTI, CRISTINA
Principal Investigator: ROBERTS, PAUL C PHD
Title: FILAMENTOUS INFLUENZA VIRUS AND MICROBIAL COLONIZATION
Institution: WAYNE STATE UNIVERSITY DETROIT, MI
Project Period: 2003/09/30-2005/09/29

DESCRIPTION (provided by applicant): Individuals recovering from influenza A virus infection demonstrate an increased susceptibility to secondary bacterial pulmonary infections, particularly with the pathogens *Streptococcus pyogenes* and *Streptococcus pneumoniae*. Quite often, these secondary bacterial pneumonias are fatal, especially in geriatric and immunocompromized patients. The mechanism(s) behind this increased susceptibility to a secondary streptococcal infection are unknown. We hypothesize that this increased susceptibility to bacterial super-infection is due to a filamentous form of influenza A virus significantly facilitating the adhesion of pathogenic streptococci to respiratory mucosa. Experiments in this application will determine if filamentous influenza A virus can directly serve as a receptor for bacterial binding or if the respiratory mucosa is altered by infection with influenza virus to promote increased bacterial adherence. In parallel, we will also determine whether prior bacterial colonization can affect influenza virus replication in respiratory epithelium.

Grant: 1R21AI054841-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: TURNER, JOANNE PHD
Title: Mycobacteria interactions and vaccine efficacy
Institution: OHIO STATE UNIVERSITY COLUMBUS, OH
Project Period: 2003/09/15-2005/08/31

DESCRIPTION (provided by applicant): Exposure to "atypical" or "environmental" mycobacteria [EM] is believed to be the primary mechanism thought to be responsible for the reduced efficacy of the BCG vaccine against tuberculosis in many parts of the world. To date however, the exact mechanism by which EM interfere with the development of protective immunity in response to vaccination still remains unknown. It has been hypothesized that constant exposure to cross-reactive antigens can sequester specific cells at alternative sites in the body, or that the immune response generated against environmental mycobacteria is strong enough to control BCG before its protective effect can be acquired. The studies documented in the literature however have used several different exposure protocols making interpretation of the results very difficult. This application seeks to carry out an analytical study using standardized exposure protocols. We propose to administer environmental mycobacteria either before BCG vaccination (to determine whether environmental exposure interferes with the generation of immunity against the vaccine) or following BCG vaccination (to determine whether environmental exposure influences the long-term efficacy of BCG). Throughout the study we will isolate immune cells from the draining lymph nodes and lungs and determine whether cells become sequestered at the site of environmental exposure, become highly activated in response to mycobacterial antigens, or become deleted from the lymphocyte pool. Using these methods we aim to determine the basis of the microbial interactions between EM and BCG that underlies the failure of the vaccine to protect against infection with *M. tuberculosis*. In addition, we propose to carry out a preliminary proteomics study to identify key antigens from *M. tuberculosis* that are absent from EM that could be incorporated into a sub-unit vaccine.

Grant: 1R21AI054847-01
Program Director: TAYLOR, KATHERINE A.
Principal Investigator: WILSON, KENNETH H MD
Title: Suppression of Clostridium difficile by Commensals
Institution: DUKE UNIVERSITY DURHAM, NC
Project Period: 2003/09/30-2005/08/31

DESCRIPTION (provided by applicant): The human colon harbors an ecosystem with a rich variety of commensal bacteria that suppress populations of pathogenic bacteria such as Clostridium difficile, the cause of antibiotic-associated colitis. C. difficile disease results when antibiotics damage bacteria that suppress this pathogen, allowing toxigenic strains to establish significant populations in the colon. Initial data from the PI's lab strongly suggest that a specific phylogenetic group of human commensals is important for the suppression of C. difficile. The goal of the work proposed in this application is to pursue further data to either support or refute the hypothesis that Clostridium coccoides and related organisms protect humans from C. difficile disease. The Specific Aims are 1) to observe the correlation between the population size of C. difficile and the size and complexity of the C. coccoides group in patients treated with antibiotics, 2) to compare the population size of C. difficile in germfree mice with that in gnotobiotic mice colonized with these human commensals and 3) to determine how these commensals suppress C. difficile in a continuous-flow culture model of the colonic ecosystem. Work related to Specific Aim 1 will involve a prospective study of patients on antibiotics. Before and after antibiotic treatment, fecal specimens will be obtained for assessment of C. difficile population size (quantitative cultures) and for determination of the composition of fecal biota. Ribosomal DNA in these specimens will be amplified with PCR, then studied by using 1) high throughput sequencing and phylogenetic analysis of obtained sequences, 2) denaturation gradient gel electrophoresis and 3) a novel 16S rDNA photolithography chip. Because there is not an adequate basis for a power calculation in Specific Aim 1, data obtained might not answer the primary question, but would allow a valid power calculation to be performed to support further study. For Specific Aim 2, germfree mice will be monoassociated with C. difficile followed by either 20 C. coccoides-related organisms, 20 C. leptum-related organisms, or no further biota. C. difficile population size will be followed by quantitative cultures. The mechanisms of suppression (Specific Aim 3) will be determined in a Freter continuous-flow culture model of the large bowel, with emphasis being placed on competition for metabolic substrates.

Grant: 1R21AI054858-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: BALABAN, NAOMI PHD
Title: Quorum sensing, bacterial interaction and disease
Institution: TUFTS UNIVERSITY BOSTON BOSTON, MA
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): The polymicrobial nature of the skin is illustrated by the isolation of both Gram-negative and Gram-positive bacteria, which altogether seem to constitute a natural barrier for opportunistic or pathogenic microbes. Bacterial cells communicate with one another by quorum sensing mechanisms, through the secretion of signaling organic molecules such as derivatives of homoserine lactones in Gram-negative bacteria and proteins and peptides in Gram-positive bacteria to activate or suppress functions necessary for their survival. As a model system for the study of the effect of quorum sensing on bacterial interaction, we will examine the effect(s) of RIP, a quorum sensing heptapeptide produced by *Staphylococcus xylosus*, on the survival and virulence of *S. aureus* and *S. epidermidis*. *S. aureus* and *S. epidermidis* are part of the normal flora of the human skin but can cause fatal diseases when their density increases, probably due to the expression of toxic exomolecules or due to the formation of biofilms. The expression of toxic exomolecules as well as biofilm formation can be inhibited by RIP, which interferes with staphylococcal quorum sensing mechanisms. RIP does not kill the bacteria but keeps them in a non-pathogenic stage, suggesting that the natural composition of the normal flora would not be effected. To further develop RIP as a therapeutic peptide, it is important to understand its molecular targets and to test its effect(s) on the normal flora in a polymicrobial environment. To achieve these goals, we propose to determine the molecular targets of RIP in *S. aureus* and *S. epidermidis* by functional genomics and proteomics and, funding and time permitting, to test its effects in vivo on the survival of a polymicrobial inoculum of both *S. aureus* and *S. epidermidis*, using the murine air sac model. Using the same in vivo model, we will also test the effect of *S. xylosus* that naturally produces RIP as a probiotic to prevent staphylococcal pathogenesis.

Grant: 1R21AI054863-01
Program Director: WALI, TONU M.
Principal Investigator: KASPER, LLOYD H
Title: Commensal Bacteria in Regulation Of T gondii Induced IBD
Institution: DARTMOUTH COLLEGE HANOVER, NH
Project Period: 2003/09/30-2005/08/31

The proposed studies investigate the interaction of *Toxoplasma gondii* and commensal bacterial flora in the immune regulation of an experimental model of pathogen-driven inflammatory bowel disease (IBD). The hypothesis to be tested is that specific bacterial products derived from commensal intestinal flora can interact with the parasite-infected host and prevent the development of experimental IBD. We have observed that germ free mice are more susceptible to an acute necrotizing condition of the ileum and colon than conventional mice following oral parasite infection suggesting that intestinal microflora or their derived products are required to prevent the development of *Toxoplasma* induced IBD. In the first specific aim, we will compare intestinal tissue samples (phenotyping, chemokines cytokines production) in germ-free strains of resistant (BALB/c) and susceptible (C57BL/6) mice following oral *Toxoplasma* infection to conventional mice. We have identified a specific capsular polysaccharide of *B. fragilis* that can modulate the gut inflammatory process. Treatment of conventional mice with capsular polysaccharide (PS A) derived from *Bacteroides fragilis* prevents the development of *T. gondii* induced IBD. The mucosal tissue and more specifically lymphoid cells from the lamina propria, Peyer's patches and other organs from PS A treated conventional and germ free mice will be assessed for immunohistologic differences. Mechanisms (IL-10, TGF- β production) of immunomodulation induced by the PS A will be further investigated. As suggested by our preliminary data, particular attention will focus on TGF- β producing CD8⁺ intraepithelial lymphocytes (IEL) and on a population of IL-10 dependent CD4 regulatory T cells (CD45RB low, CD25). We will evaluate for the expression of these regulatory CD4⁺ T cells in the lamina propria and Peyer's patches of PS A treated mice and determine whether this cell population can be adaptively transferred to naive mice and prevent toxoplasma-driven IBD. NKT cells are also important regulatory cells that respond to polysaccharides. We will evaluate whether PS A treatment can induce a population of NKT cells that exert effector and immunoregulatory activity.

Grant: 1R21AI054875-01
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: BYRNE, GERALD I PHD
Title: Chlamydia trachomatis, Genital Flora, & STD Pathogenesis
Institution: UNIVERSITY OF TENNESSEE HEALTH SCI MEMPHIS, TN
CTR
Project Period: 2003/03/15-2005/02/28

DESCRIPTION (provided by applicant): Chlamydial genital tract infections in women continue to be a huge public health problem in the United States and across the globe. These infections also can become personal tragedies for infected individuals since they frequently lead to upper genital tract sequelae that can result in involuntary infertility due to tubal scarring and occlusion. *C. trachomatis* genital tract infections in women also occur in a polymicrobial environment, but little information is available on how the female genital flora affects disease outcome caused by Chlamydiae. Recent studies demonstrate that *C. trachomatis* genital isolates differ from ocular strains in that the former have functional tryptophan biosynthetic genes, whereas ocular strains do not. Chlamydial strains that have the capacity to produce tryptophan may be at a distinct advantage in the in vivo environment, since a major chlamydial antimicrobial effect elicited by host immune reactivity is manifest in chlamydial host cells through the induction of a tryptophan decylcizing enzyme (indoleamine 2,3-dioxygenase, [IDO]) after activation by the CD4+, Th1 effector cytokine, gamma interferon. The tryptophan operon in genital *C. trachomatis* isolates, however, is incomplete in that indole or indole derivatives are required for tryptophan production by these strains. Indole is produced neither by Chlamydiae nor the human host, and the hypothesis to be tested in this application is that normal indole-producing genital flora contribute to Chlamydiae disease pathogenesis by producing the substrate necessary for *C. trachomatis* to survive and grow in the lower genital tract and thereby contribute to improved transmission potential as well as the possibility for more severe upper genital tract consequences. This hypothesis will be tested by determining the effects of indole-producing normal flora on chlamydial genital tract disease in a well-characterized murine model.

Grant: 1R21AI054891-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: MONTANER, LUIS J PHD EXPERIMENTAL
PATHOLOGY
Title: Immune Determinants to Bacterial and Viral Co-Infection
Institution: WISTAR INSTITUTE PHILADELPHIA, PA
Project Period: 2003/01/01-2004/12/31

DESCRIPTION (provided by applicant): The long-range goal of this application is to determine the factors that predict the manner in which pathogenesis develops during poly-microbial infections. The short-term goal of this project will be to determine the manner in which BCG-associated inflammation and its modulation of antigen presenting cells affects a new immune response to a vaccine antigen delivered as and inactivated organism. Our preliminary studies support our goals and application by establishing a link between innate DC dysfunction and lower adaptive responses to vaccine antigens delivered as an inactivated organism during an ongoing bacterial infection. Based on our preliminary observations, we hypothesize that a decreased potential to develop protective immune responses during Mycobacterial infection is due to a cyclic period of down-regulation of accessory cell function and a decrease of CD11c cell subsets. We will test this hypothesis by defining immune correlates and gene expression patterns within CD11c+ and CD11b+ accessory cell subsets during BCG infection through [1] longitudinal analysis of the changes in B-cell proliferation, T-cell activation, DC cell subsets, DC activation (CD86, CD80, CD40, CD95, MHC-II) and function (MLR, endocytosis, TLR-4 induced IL-12p70, IL-10, TNF- α secretion), and RNA gene expression of sorted CD11c+CD11b+ or CD11c+CD11b+ DC subsets from longitudinal time points by cDNA microarrays of un-stimulated and in vitro stimulated cultures; and [2] establishing the biological impact of accessory cell changes due to primary BCG infection on the development of secondary anti-flu responses by analysis of the development and recruitment of antiviral humoral and cell-mediated immune memory responses acquired through UV inactivated Influenza A/PR8 vaccination of naive or BCG-infected animals at weekly intervals throughout BCG infection. We apply a vaccine approach within an on-going bacterial infection as a surrogate method to elicit a primary immune response and its associated memory pools with minimal pathology or additional pathogen co-factors. We will compare morbidity and mortality outcomes to developing antiviral immune responses following live challenge of animals having received vaccination against Influenza A/PR8 at different periods of BCG infection and clearance. Completion of this application will provide identify innovative targets for increased susceptibility to bacterial/viral co-infections by addressing understudied areas of innate immunity and chronic inflammation as central factors to decreased adaptive responses and protective immunity. This application represents a collaborative effort by The Wistar Institute and the Department of Dermatology, and the Center for Clinical Epidemiology and Biostatistics from the University of Pennsylvania.

Grant: 1R21AI054892-01
Program Director: SCHMITT, CLARE K.
Principal Investigator: CRANE, JOHN K
Title: Enteropathogenic and Toxigenic E.coli Interactions
Institution: STATE UNIVERSITY OF NEW YORK AT AMHERST, NY
BUFFALO
Project Period: 2003/09/30-2005/08/31

DESCRIPTION (provided by applicant): Enterotoxigenic Escherichia coli (ETEC) and enteropathogenic E. coli (EPEC) are common causes of diarrhea among children in developing countries. ETEC is also the most common cause of traveler's diarrhea in adults. ETEC strains produce either the heat-labile toxin, L T, or heat-stable toxin, ST (also called STa) or both. EPEC does not produce any known toxins, and the way that it triggers diarrhea is still not clear. ETEC and EPEC are commonly isolated together as dual infections in the same patient. The discovery that EPEC triggers host cell death provided an important lead in how EPEC causes disease. The mode of cell death triggered by EPEC has features of both apoptosis (programmed cell death) and necrosis. One of the non-apoptotic features of EPEC-mediated killing is release of adenosine triphosphate (ATP) from the host cell. Once released, ATP is broken down to ADP, AMP, and adenosine. Adenosine itself acts as a potent secretagogue, i.e, a stimulator of intestinal fluid and electrolyte secretion, which may cause or contribute to watery diarrhea. Results from the applicant's laboratory show that cellular signaling events triggered by EPEC specifically increase the activity of ETEC STa toxin via EPEC-mediated changes in protein kinase C (PKC) and tight junctions. Furthermore, the E. coli LT toxin potentiates the ATP release triggered by EPEC infection. Therefore, ETEC and EPEC are able, at least in vitro, to mutually enhance the virulence of the other. The goals of this application are to understand molecular mechanisms by which dual infections with ETEC and EPEC generate "cross-talk" between signaling pathways in the host, and how this cross-talk is coupled to increased secretion of fluid and electrolytes in the intestinal tract.

Grant: 1R21AI054893-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: SANDOR, MATYAS PHD
Title: Secondary infections during mycobacterial disease
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 2003/09/30-2005/08/31

DESCRIPTION (provided by applicant): Approximately 30% of the world's population has been infected with *Mycobacterium tuberculosis*, and tuberculosis has been estimated to be the leading cause of death worldwide. A significant fraction of that mortality is from weakened cellular immunity due to co-infection with other endemic infections. Anti-mycobacterial immunity is mediated primarily by CD4+ T cells, which regulate the formation of protective granulomatous inflammation to restrict pathogen dissemination and protect surrounding healthy tissue. We propose to use a mouse model of chronic tuberculosis, *M. bovis* strain bacille Calmette Guérin (BCG), to examine the effect of super-infection with influenza virus and the fungus *Histoplasma capsulatum*. We will genetically mark both the BCG and secondary pathogen with different well-characterized T cell epitopes. Using epitope specific class II tetramer staining reagents, we can follow the localization and phenotype of TCR-populations specific for either BCG or the secondary pathogen by flow cytometric analysis of granuloma cells. This model will also be used to examine the effect of super-infection upon organ load, the capacity of different TCR-specific T cell populations to produce protective cytokines, and gene-expression changes associated with local antigenic specificity. T cell interactions (help, suppression, competition) have been described with nominal antigens, and our studies will focus on how T cell responses for two infectious agents interfere with each other. We intend to make our observations by infecting animals with a full spectrum of T cells (B10.BR), infecting B10.BR animals into which TCR transgenic T cell populations specific for both epitopes have been adoptively transferred as sentinels, and infecting immunodeficient Rag2^{-/-} after adoptive transfer of TCR transgenic T cells that are specific for the epitope tags expressed by the infectious agents to construct a small T-cell network. Thus we can very precisely study the recruitment and functional qualities of granuloma cells having mycobacterial specificity and those with specificity for other infections. The goal of this research is to understand the interaction of T cell responses controlling different infections. Hopefully, this will lead to better treatments of mycobacterial disease.

Grant: 1R21AI054897-01
Program Director: RUBIN, FRAN A.
Principal Investigator: PRITCHARD, DAVID G PHD
Title: Inhibition of GBS Carriage by Engineered Lactobacilli
Institution: UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL
BIRMINGHAM
Project Period: 2003/02/01-2005/01/31

DESCRIPTION (provided by applicant): Group B Streptococci (GBS) are a major cause of serious neonatal bacterial infections in this country. The origin of the bacteria for babies born with early-onset GBS infections is the birth canal of their mothers. This application describes an entirely new approach for preventing or eliminating vaginal carriage of GBS, thereby reducing the incidence of neonatal GBS infections. The goal is to genetically engineer a normal commensal organism present in the vagina, a Lactobacillus, to secrete a substance that will specifically prevent the growth of GBS. The substance selected is a GBS phage lysin that degrades the cell walls of GBS. The first specific aim is to clone and express the GBS phage lysin in E. coli and assess the bacteriocidal activity of purified recombinant enzyme. The ability of the purified lysin to kill GBS and various other bacteria will be first studied in vitro. Then it will then be determined if vaginal instillation of the enzyme will eliminate GBS in vaginally colonized mice. The second specific aim is to determine basic biochemical properties of the GBS phage lysin, including identifying the enzyme class to which it belongs and the identity of the cell wall component(s) necessary for specific peptidoglycan cleavage. The third specific aim is to engineer a Lactobacillus to secrete the GBS phage lysin. This will be done initially using a plasmid construct containing all the elements necessary for efficient secretion of the lysin from the transformed Lactobacillus. Then, in order to overcome inherent plasmid instability and also to eliminate the antibiotic selection markers, the lysin gene secretion cassette will be integrated into the chromosome of the Lactobacillus. The final specific aim is to determine if vaginal colonization of mice with the engineered Lactobacillus will prevent GBS from establishing a persistent colonization. In addition, it will also be determined if vaginal inoculation with the lactobacillus engineered to secrete GBS phage lysin will result in the clearance of GBS from the vaginas of mice previously colonized with the organism. The proposed research may lead to the development of an effective new method for long-term inhibition of GBS vaginal colonization, even though women may be repeatedly re-exposed to the bacteria. The method is unlikely to disturb the normal bacterial flora and should be very safe. If successful, this approach might also be used to protect against other genital, oral, and intestinal pathogens, especially those to which no effective mucosal immunity appears to develop.

Grant: 1R21AI055013-01
Program Director: ZOU, LANLING
Principal Investigator: CLEMENTS, JOHN D
Title: Combinatorial vaccines against anthrax and plague
Institution: TULANE UNIVERSITY OF LOUISIANA NEW ORLEANS, LA
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): Until recently, anthrax was primarily a concern of individuals working in animal husbandry and military planners concerned about the potential use of anthrax spores as an agent of biological warfare. The use of anthrax as an agent of bioterrorism on civilian populations was a theoretical risk, heightened by the discovery that certain rogue nations (notably Iraq, Syria, and China) either had developed or were attempting to develop anthrax or other biologic agents as a weapon of mass destruction. The post- September 11 release of anthrax spores resulted in five civilian deaths, eighteen infections, and required that more than 30,000 individuals to undergo prophylactic antibiotic therapy. This event also highlighted the need for improved vaccines that would be appropriate for pre- or post- exposure immunization of civilian and military populations against potential bioterrorism agents, including anthrax and plague. Vaccines combining protective antigens from different microorganisms with potential for use against civilian or military populations as biological warfare/biological terrorism agents would be advantageous because they would both broaden the coverage of such vaccines and reduce the overall number of immunizations. The first logical combination to examine would be rPA from *B. anthracis* and F1-V from *Y. pestis* since they have individually been shown to induce protective responses. Combining vaccines to decrease the number of immunizations and to increase vaccine coverage is not a new concept in vaccine development and combination vaccines such as DTP and MMR have been used for many years. However, several examples of immunologic interference between individual components of combination vaccines have been observed both in clinical trials and in laboratory tests. We are therefore proposing to examine the potential of a combined vaccine consisting of rPA and F1-V with the specific objective of determining synergy or interference between the vaccine components. For this application, we propose to address a number of interrelated questions regarding immunization with a combined vaccine containing rPA and F1-V as immunogens to protect against anthrax and plague.

Grant: 1R21AI055284-01
Program Director: NEAR, KAREN A.
Principal Investigator: HEIFETS, LEONID B MD
Title: Nanoparticle delivery system for antituberculosis drug
Institution: NATIONAL JEWISH MEDICAL & RES CTR DENVER, CO
Project Period: 2003/05/01-2005/04/30

DESCRIPTION (provided by applicant): The U.S. Department of State recently invited us to collaborate with Russian Research Center of Molecular Diagnostics and Therapy (RDCMDT) in the evaluation of nanoparticles as drug delivery vehicles for anti-tuberculosis drugs. While the project by RDCMDT is funded through the International Science and Technology Center (ISTC), funding for the portions of the project to be performed at National Jewish must be funded from U.S. domestic sources. Nanoparticles are small (Submicron) colloidal particles in which different drugs can be entrapped or adsorbed, and their usefulness as an advanced drug delivery system has been demonstrated in many areas. They can be stored without refrigeration for extended periods, and may enable targeting of anti-tuberculosis drugs to macrophages, and the intracellular compartments of macrophages in which Mycobacterium tuberculosis resides. We hypothesize that formulations of existing antituberculosis drugs within nanoparticles modified to traffic to intracellular compartments bearing M. tuberculosis will result in a reduction of the minimal inhibitory concentrations (MIC) relative to the free drugs. Our preliminary data, the only data on anti-TB drugs, suggest that the MICs for streptomycin and isoniazid were reduced by 4.5-7 fold. Dr. Geuelperina at RDCMDT has already begun encapsulation of ethambutol, pyrazinamide, capreomycin, amikacin, kanamycin, ethionamide, levofloxacin, cycloserine, moxifloxacin, vancomycin, and ceftiofloxacin. These drugs, if efficiently encapsulated, will be tested in a macrophage model of M. tuberculosis infection at National Jewish Medical and Research Center in Denver. We will test the hypothesis that increasing the intracellular accumulation, particularly in phagosomes harboring M. tuberculosis will result in enhanced efficacy, as measured by reduced growth and viability of intracellular organisms. We will characterize the accumulation of each formulation by labelling the drugs and following their routes of uptake when the particles are opsonized by serum proteins, when opsonization is inhibited by coating the particles with polyethylene glycol, and when the nanoparticles are targeted to specific cell surface receptors via modification with transferrin or mannoside clusters. Distribution of the drugs within the cells over time will also be monitored by immunostaining of the drug molecules within the cells at increasing intervals following uptake. The immunostaining will be made quantitative by creating standard curves for each drug concentration, such that concentrations within particular cellular compartments can be estimated. Finally, we will correlate viability of intracellular M. tuberculosis with drug exposure at the sub cellular level, and determine which formulations are most effective at directing drug accumulation in the cellular compartments harboring M. tuberculosis. Successful completion of this project will provide us with the most promising formulations of encapsulated anti-TB drugs which will create a basis for further collaboration with RDCMDT in preclinical development, including animal models of efficacy, pharmacokinetics, and toxicology.

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Grant: 1R21AI055344-01A1
Program Director: LAMBROS, CHRIS
Principal Investigator: LI, RONGBAO PHD
Title: Novel Antifolates against AIDS-associated Tuberculosis
Institution: SOUTHERN RESEARCH INSTITUTE BIRMINGHAM, AL
Project Period: 2003/08/01-2005/07/31

DESCRIPTION (provided by applicant): The overall goal of this project is to develop both potent and selective antifolates against *Mycobacterium tuberculosis* (Mtb), an opportunistic pathogen known to cause morbidity and mortality in AIDS patients. Specifically this project will focus on design of novel antifolates, through a structure-based approach in light of the recently solved crystal structure of Mtb dihydrofolate reductase (DHFR), organic synthesis, and evaluation of the synthetic compounds for their biological activity. DHFR is a key enzyme of the folate metabolic pathway and is required for both prokaryotic and eukaryotic cell-growth. Recent clinical studies of agents inhibiting enzymes of the folate pathway, including DHFR, demonstrated therapeutic effect in AIDS-associated TB patients. Highly potent DHFR inhibitors are available but are toxic due to their low selectivity. To increase the selectivity, a new pharmacophore model is needed, which relies critically on structural differences between the host and pathogen enzymes. The comparison between the available structures of the host and the pathogen DHFR enzymes has revealed potential target sites that allow for the design of selective inhibitors. With the common binding motifs of the DHFR family as platforms, such as 2,4-diaminopyrimidine and diaminopteridine heterocyclic systems, we have designed a series of molecules with special features that would bind tightly and specifically to these sites on Mtb DHFR but unlikely to the same sites on the human enzyme. In this proposed research, these designed molecules will be synthesized and evaluated for their inhibition of DHFR and their effect on Mtb cell growth. Lead compounds that actively and selectively inhibit Mtb will be co-crystallized with Mtb DHFR. The crystal structures of these complexes will be determined by X-ray crystallography. The structural analysis of these complexes will reveal the ligand binding mode to the target and the structural changes in the target induced by the binding of these compounds, which is critically useful for the lead-compound optimization. This study presents a new direction in the design of antifolates against mycobacterial infection. This study will also provide the molecular basis for further developing compounds that are highly potent and selective against Mtb and are potentially useful for the TB treatment.

Grant: 1R21AI055487-01
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: HASSETT, DANIEL J PHD
Title: Proteogenome of Anaerobic *P. aeruginosa* in CF Mucus
Institution: UNIVERSITY OF CINCINNATI CINCINNATI, OH
Project Period: 2003/04/15-2005/03/31

DESCRIPTION (provided by applicant): CF patients succumb to pulmonary insufficiency, resulting from complications due to intractable *P. aeruginosa* biofilms. Because there is no animal model for CF airway disease, in vitro biofilm studies have been performed using a variety of substrata (plastic, glass, stones, etc.) for bacterial attachment and biofilm development. However, unlike abiotic surfaces or even cells, CF airway bacteria are not associated with the epithelium. Instead, they are enmeshed in hypoxic or anaerobic mucopurulent masses in thick mucus lining the airway lumen. Therefore, we hypothesize that *P. aeruginosa* is undergoing anaerobic respiration (denitrification) during the course of CF airway disease and the process of denitrification is likely critical for optimal growth and survival of the organism in CF airway mucus. Several lines of evidence suggest that these hypotheses are correct. First, antibodies are raised by CF patients against proteins involved in anaerobic metabolism of *P. aeruginosa*. Second, *P. aeruginosa* alginate production, that is directly associated with CF morbidity and mortality, and is typically unstable in vitro. Yet it is stabilized during anaerobic growth in the presence of the alternative electron acceptor, nitrate, that is present in ample quantities in CF airway mucus for anaerobic growth of *P. aeruginosa*. With the completed *P. aeruginosa* genome and the development of genomics/proteomics, a timely opportunity exists to monitor whole-genome transcription and protein expression patterns under defined conditions that are relevant to the pathogenesis of CF airway disease. The research agenda for this proposal will include a set of experiments using 1- and 2-D gel/MALDI mass spectrometry/proteomics, capillary LCMS mass spectrometry (whole-bacterium proteome analysis), and microarray transcriptional profiling that are organized to fulfill a single primary goal - to identify *P. aeruginosa* genes and proteins necessary for optimal survival of biofilm bacteria undergoing anaerobic metabolism in mucus derived from CF primary cell lines. The genes/proteins identified in these studies are predicted to mimic those expressed in various stages of CF airway infection. Our LONG TERM GOAL is to identify cellular targets that inhibit or significantly compromise the denitrification process that is occurring by *P. aeruginosa* in the CF airways.

Grant: 1R21AI055551-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: PAVELKA, MARTIN S BS
Title: Development of genetic tools for Francisella tularensis
Institution: UNIVERSITY OF ROCHESTER ROCHESTER, NY
Project Period: 2003/08/01-2005/07/31

DESCRIPTION (provided by applicant): The Gram-negative bacterium *Francisella tularensis* subsp. *tularensis*, the causative agent of tularemia, is considered a potentially dangerous biological weapon. This is due to the organism's extreme infectivity via the aerosol route and the severity of the human disease it causes. The fastidious nature of the bacterium, the requirement that it be handled using biosafety level three (BSL3) containment, and the paucity of genetic tools have hindered research on this organism. Thus, compared to other pathogens, little is known about *F. tularensis* subsp. *tularensis* physiology, genetics, and pathogenesis. Previous studies have used the attenuated live vaccine strain (LVS) derived from *F. tularensis* subsp. *palaeartica*, or the rarely pathogenic *F. tularensis* subsp. *novicida*. The aim of this proposal is to develop genetic tools for the manipulation of virulent, pathogenic *F. tularensis* subsp. *tularensis*. These tools will then be used for the analysis of *F. tularensis* subsp. *tularensis* pathogenesis. Furthermore, the ability to genetically manipulate the organism will allow for the development of attenuated vaccine strains that could be administered to populations at risk from *F. tularensis* infection, either from natural sources or from a biological attack. The further development of *Francisella* genetics is timely, as the sequencing of the genome of the *F. tularensis* subsp. *tularensis*, strain Schu 4 is almost complete.

Grant: 1R21AI055618-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: FIKRIG, EROL MD MEDICINE
Title: Neutrophil gene expression induced by *A. phagocytophila*
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 2003/08/01-2005/07/31

DESCRIPTION (provided by applicant): This application is a supplement to our recent proposal entitled "Immunopathogenesis of granulocytic Ehrlichiosis" (R01 A1041440), in which we proposed to understand the pathogenesis of HGE using both in vitro and in vivo models. Our recently published report demonstrates that *A. phagocytophila* inhibits the respiratory burst by selectively down-regulating gp91 phox, a major subunit of the NADPH oxidase holoenzyme. The proposed R21 supplement represents an opportunity to use innovative technologies to expand the scope of our current R01 and its pending renewal. We have established a collaboration with Dr. Nancy Berliner's laboratory that offers a unique opportunity to broaden our understanding of the changes in neutrophil biology induced by *A. phagocytophila* infection. Collaboration with her laboratory provides complementary expertise to our own, since her work has focused on the transcriptional regulatory events governing normal myeloid stem cell maturation. Dr. Nancy Berliner and her colleagues offer expertise in gene expression profiling, chromatin immunoprecipitation, and structure-function analysis of transcription factors that will apply to the proposed studies. In these supplemental studies we will assess and characterize the changes in global gene expression associated with *A. phagocytophila* infection using a myeloid-specific microarray recently described by Dr Berliner and her colleagues. We will also use chromatin immunoprecipitation (CHIP) and structure-function analyses to further explore the role of CCAAT displacement protein (CDP) in the context of its role in the neutrophil differentiation program and how it is altered by *A. phagocytophila* infection. As outlined in our R01 proposal, we hypothesize that the repression of gp91 phox by *A. phagocytophila* is mediated by CDP, a transcriptional repressor of many granulocytic genes. Dr. Berliner and her colleagues have characterized the role of aberrant CDP binding in repressing late neutrophil-specific gene expression in leukemia cell models, and are ideally poised to perform similar studies in concert with our experiments. The proposed studies in combination with those outlined in R01 A1041440 promise to lead to a greater understanding of HGE and the molecular mechanisms that *A. phagocytophila* uses to interfere with neutrophil differentiation and function

Grant: 1R21AI055627-01
Program Director: TSENG, CHRISTOPHER K.
Principal Investigator: DE LA TORRE, JUAN C PHD VIROLOGY,
MOLECULAR BIOLOGY
Title: Aminoglycoside Antivirals to Combat Arenaviruses
Institution: SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA
Project Period: 2003/08/01-2005/07/31

DESCRIPTION (provided by applicant): The prototypic arenavirus lymphocytic choriomeningitis virus (LCMV) is an excellent model to study the molecular and cellular biology of arenaviruses that cause important and severe human diseases like Lassa fever virus (LFV) and the South American viral hemorrhagic fevers (SAHF). In addition, weaponized forms of these viruses pose a real threat as agents of bioterrorism. No licensed vaccine is available in the US, and currently there is not efficacious therapy to treat these viral infections. Therefore the importance of developing novel effective antiviral drugs to combat pathogenic arenaviruses. We have developed a reverse genetic system for LCMV. This system provides us with a robust platform for the development of novel strategies to target specific steps of the Arenavirus life cycle. We have molecularly characterized the Arenavirus genome promoter. Disruption of the interaction between the viral polymerase and promoter is predicted to abrogate virus multiplication. RNA molecules can form intricate structures that can be targeted by selected specific high-affinity antagonists. This proposal will explore the use of aminoglycoside-based small molecules to target and functionally disrupt the Arenavirus genome promoter, thus inhibiting virus infection. Aim 1 is the screening of combinatorial aminoglycoside libraries to identify small molecules that bind to the LCMV genome promoter. For this, biotin-RNA conjugates of the LCMV promoter will be attached to streptavidin-coated sensor chips and used as a ligand to screen the libraries using surface plasmon resonance. Aim 2 will assess the antiviral activity of binder molecules (Aim 1). Selected aminoglycosides will be tested for: (i) their effects on RNA synthesis mediated by the LCMV polymerase using our LCMV minigenome system; (ii) their ability to inhibit LCMV multiplication in cultured cells. Finally, Aim 3 will assess the generation of viral variants resistant to antiviral aminoglycosides. Emergence of resistant variants will be assessed based on production of infectious virus and intracellular levels of virus RNA synthesis during serial passages in the presence of aminoglycosides with anti-LCMV activity.

Grant: 1R21AI055643-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: BARROW, WILLIAM W PHD MICROBIOLOGY, OTTAWA
Title: Narrow-Spectrum Drug Targets for Bacillus anthracis
Institution: OKLAHOMA STATE UNIVERSITY STILLWATER, OK
STILLWATER
Project Period: 2003/09/30-2005/08/31

DESCRIPTION (provided by investigator): This application is in response to NIAID's request for high-priority biodefense products specific to CDC Category A-C Priority Pathogens. It focuses on the development of compounds with selective activity against Bacillus anthracis, included in the highest priority group, Category A. The goal of this application is to show proof-of-principal for potential antimicrobial targets in a specific biosynthetic pathway of Bacillus anthracis that will allow for the development of compounds that can be used to treat anthrax. At least two enzymes in this pathway represent targets that are naturally resistant to standard antimicrobial agents. As a result, traditional drugs for these targets are currently not recommended for treatment of anthrax. We hypothesize that these targets are amenable to new drug development and that they may be susceptible to non-traditional antimicrobials that may already exist in chemical repositories. Consequently, one goal of this project will be to implement the most expedient way to screen drugs that are already available against these enzyme targets. Because the degree of homology of these enzymes is so high, we hypothesize that B. cereus can be used in place of Bacillus anthracis. This will facilitate the use of high-throughput screening with a robotics system in a BSL-2 versus a BSL-3 facility. The approach will be to use DNA recombinant technology coupled with X-ray crystallographic molecular modeling to demonstrate proof-of-principal with regard to functional similarities of two key enzymes in an essential metabolic pathway of Bacillus anthracis. Recombinant enzymes will be used in drug-screening assays and for crystallization to develop effective three-dimensional models to confirm enzyme similarities. If successful, this strategy would prove useful for other enzymes in this pathway that also share a high degree of homology. This R21 application is intended to lay the groundwork for a subsequent RO1 that will be designed to attain narrow-spectrum antibiotics for Bacillus anthracis through early product development resulting from collaborations between academic researchers and industrial laboratories.

Grant: 1R21AI055645-01
Program Director: BEANAN, MAUREEN J.
Principal Investigator: PETRENKO, VALERY A PHD
Title: Phage Binding for Continuous Anthrax Spore Detection
Institution: AUBURN UNIVERSITY AT AUBURN AUBURN UNIVERSITY, AL
Project Period: 2003/08/01-2005/07/31

DESCRIPTION (provided by investigator): Spores of *Bacillus anthracis* (BAS) poses a major bioterrorism threat because of their extreme potency and availability. There is an urgent need to rapidly detect BAS for efficient treatment of anthrax. The ultimate goal of this application is the development of a device that could be mounted to a SpinCon (air-to-liquid) concentrator for the continuous monitoring, detection and alarm of the presence of airborne anthrax spores. Fluid from the SpinCon would be directed across the surface of the Auburn University-designed sensor, which could continuously monitor for the presence of anthrax spores. Detection would occur by the binding of the anthrax spores to phage-derived probes (BAS probes) specifically designed to target these spores. BAS-probes will be selected from the billion-clone phage libraries expressing foreign random peptides on all 4,000 copies of the viral major coat protein (landscape libraries), and will be improved using mutagenesis and affinity maturation. The phage particles demonstrating best binding to BAS in Enzyme Linked Immuno Sorbent Assay (ELISA) will be remodeled to eliminate their *Escherichia coli*-binding domains, and will be immobilized onto the surface of either a miniaturized plasmon surface resonance or acoustic wave resonator to produce a signal indicating the detection of anthrax spores. Specificity and selectivity of detection of BAS will be studied using BAS mixed with unrelated spores, proteins and other biopolymers. Parallel experiments will also be conducted with antibody-derived probes for comparison evaluation of specificity, selectivity and longevity of the sensors.

Grant: 1R21AI055657-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: GREGORY, STEPHEN H
Title: FRANCISELLA TULARENSIS: INNATE RESISTANCE TO IHALATION
Institution: RHODE ISLAND HOSPITAL (PROVIDENCE, PROVIDENCE, RI
RI)
Project Period: 2003/09/15-2005/08/31

DESCRIPTION (provided by applicant): Francisella tularensis is one of the most infectious pathogens known to man, as few as 10 organisms can cause disease. Aerosolized F. tularensis represents a potentially dangerous biological weapon. Alveolar macrophages (AM) constitute both the principal target for intracellular replication and a key effector for the elimination of F. tularensis deposited in the lungs. Infiltrating neutrophils are a prominent feature of pneumonic tularemia. Factors judged important in host defenses to F. tularensis are based largely upon studies involving mice inoculated intravenously. The role of specific defense mechanisms varies, however, dependent upon the route of infection (i.e., inhalation vs. systemic) and primary site of invasion (lung vs. spleen or liver). Recent studies conducted in our laboratory indicate that the interaction between inflammatory neutrophils and resident tissue macrophages plays a critical role in host defenses to bacterial infections expressed in the liver. Similarly, it is speculated here that neutrophil-AM interaction exerts a significant influence on innate resistance to F. tularensis in the lungs. The SPECIFIC AIMS of this proposal are to: I. Delineate the biological response of AM to aerosolized F. tularensis, i.e., IA. quantify phagocytosis of F. tularensis, apoptosis and the antimicrobial activity of AM, IB. quantify cytokine and chemokine production, IC. assess the intermediary role of AM in the infiltration and bactericidal activity of neutrophils, and ID. define the role of AM in down-regulating neutrophils sequestration and tissue injury. II. Determine the effect of infiltrating neutrophils on proinflammatory cytokine and chemokine production by AM. Given the limited number of relevant studies to date, the results of the proposed experiments should dramatically increase our current understanding of the factors that effect innate host defenses to pulmonary F. tularensis infections. This, in turn, should enable the development of innovative strategies to improve treatment and prevent the fatal consequences that often occur in untreated cases of pneumonic tularemia. Moreover, our primary approach to studying cytokine gene expression by inflammatory cells (i.e., immuno-laser capture microdissection followed by GeneChip expression microarray analysis of mRNA) should afford considerable insight into the factors, pathways and underlying mechanisms that effect innate immunity to respiratory infections in general.

Grant: 1R21AI055713-01
Program Director: TAYLOR, KATHERINE A.
Principal Investigator: WEISSMAN, SHERMAN M. MD OTHER CL
MED:CLINICAL
MEDICINE,UNSPEC
Title: Global Scanning for Resistance Mutations in H.pylori
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 2003/09/01-2005/08/31

DESCRIPTION (provided by applicant): *Helicobacter pylori* (Hp) is a major cause of peptic ulcer disease and an early risk factor for gastric cancer. Metronidazole (Mtz) is an antibacterial agent used against Hp, but resistance is common and is a major reason for treatment failure. Research by D. Berg has shown that this resistance is a polygenic trait and that null mutations in the gene *rdxA* confers low-level resistance; a mutation in another gene, *frxA*, results in higher resistance and that higher resistance (hyper-resistance) results from mutations in another genes that remain to be identified (Jeong et al. J. Bact. 182:3219, 2000). We [Pan and Weissman PNAS: 99:9346 (2002)] have developed a method for global screening for mutations in complex DNA mixtures. In the present application we propose to optimize and apply this method for analysis of bacterial genomes, and use the method to identify mutations and genes that make Hp hyper-resistant to Mtz. The final purpose of this project is to provide a general research tool for dissecting the molecular mechanisms of bacterial pathogenicity and genome evolution. These experiments will help meet Specific Aim 4 of parent grant AI38166 to DE Berg (co-PI) [To more fully understand mechanisms of drug resistance]. This project is "exploratory" or "developmental," relative to the parent grant, in that we may need to overcome potential "noise" from duplicate and divergent sequences as well as other unexpected problems, and generally optimize this for microbial systems. Nevertheless, this method should allow identification of the genes responsible for hyper-resistance with an efficiency that could not be matched by traditional (e.g. shotgun cloning and DNA transformation) type protocols, especially if different constellations of genes cause hyperR in different strains (genetic backgrounds).

Grant: 1R21AI055752-01
Program Director: KLEIN, DAVID L
Principal Investigator: LIETMAN, THOMAS M MD
Title: Predicting Resistance: Validating Mathematical Models
Institution: UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA
Project Period: 2003/09/30-2005/09/29

DESCRIPTION (provided by applicant): There is concern that antimicrobial resistance is spreading worldwide, seriously limiting our ability to successfully treat a wide variety of diseases. Mathematical models have contributed much to our understanding of drug resistance, but their results have not been widely accepted in clinical medicine, perhaps because of parameter uncertainty and lack of validation. Mass antibiotic distributions are currently being implemented around the world as part of the WHO's effort to eliminate trachoma, the leading cause of infectious blindness. These distributions offer an unprecedented opportunity to model the emergence of antimicrobial resistance and to test the model's predictions empirically. Programs know precisely who is treated, when they are treated, and what dose of antibiotic is taken. In this application, we develop mathematical models to predict how much resistance will emerge after multiple rounds of mass antibiotic administrations, and then test the models' predictions by collecting data from the field. We anticipate that this project will evaluate several principles of resistance modeling and evaluate the validity of mathematical transmission models in general. Specific Aims: 1. To determine the strength of the association between macrolide use and macrolide-resistant pneumococcus using data from existing epidemiological studies. 2. To estimate the level of resistance following mass azithromycin distributions for trachoma with mathematical models. 3. To test the validity of mathematical models by determining the prevalence of pneumococcal resistance empirically after multiple mass distributions.

Grant: 1R21AI055756-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: ADAMS, JULIAN P PHD GENETICS:GENETICS
EVOLUTION
Title: Ciprofloxacin resistance and compensatory mutations
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): The evolution of antibiotic resistance in human pathogenic microorganisms is increasingly becoming a serious public health problem. A number of measures have been proposed to combat the spread of antibiotic resistance with varying success, including controls of the use of antibiotics, the use of vaccines and improvement of hospital hygiene. However, the ultimate success of such measures is questionable, due to evolutionary changes in the resistant sector of the microorganism populations. Many (but not all) mutations to antibiotic resistance are deleterious, and therefore may be selected against in the absence of the antibiotic. However, under prolonged exposure to antibiotics, compensatory mutations can and do occur and be selected, which reduce the cost associated with antibiotic resistance. Such evolutionary changes will contribute to the prevalence to antibiotic resistant pathogens, and render some antibiotics ineffective. Such a scenario will be particularly disastrous during an anthrax epidemic in this country, for which ciprofloxacin will be the primary antibiotic of choice. The principal focus of this project will be to characterize and identify compensatory mutations, which reduce the cost of ciprofloxacin resistance in *B. subtilis*, a close relative of *B. anthracis*. The study will also be extended to include *E. coli* and two other antibiotics, novobiocin and ceftazidime. Knowledge of the biochemical and physiological basis of compensatory mutations may allow the design of strategies to reduce or counteract their role in increasing the prevalence of antibiotic resistant mutations.

Grant: 1R21AI055760-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: KNAPP, SPENCER PHD
Title: Inhibition of Tubercular Mycothiol Pathways
Institution: RUTGERS THE ST UNIV OF NJ NEW BRUNSWICK, NJ
BRUNSWICK
Project Period: 2003/04/15-2005/03/31

DESCRIPTION (provided by applicant): Drug-resistant tuberculosis now threatens a large portion of the earth's population, and the development of new treatments for tuberculosis infection has become a national and international priority. Mycobacterium tuberculosis depends on a low molecular weight thiol, "mycothiol," to maintain a reducing intracellular environment and to remove exogenous electrophilic agents. Disruption of the enzymatic pathways of mycothiol biosynthesis and/or mycothiol-based detoxification could leave M. tuberculosis vulnerable to drugs, oxygen, and other stress factors, and constitutes a new tactic for the control of tuberculosis. The objective of this project is to develop inhibitors of the mycothiol-related enzymes of M. tuberculosis, and eventually to design new and successful treatments for tuberculosis. Three enzymes will be targeted initially: mycothione reductase, mycothiol S-conjugate amidase, and inositol GlcNAc deacetylase, although others, including a cysteine ligase and a cysteine transacetylase, could be added. This work will be guided by enzymatic assays conducted by collaborators using existing screens, and by preliminary results that already indicate that substantial structural simplification in designing mycothiol analogues is possible. First, the minimum substrate requirements for the M. tuberculosis enzymes will be defined. Then, inhibitors based on these minimum structures will be synthesized and evaluated. New methods for the synthesis of mycothiol-analogous compounds will be developed, and new ideas for enzyme inhibitor design will be explored. The most active compounds will be taken as leads for further analogue development and for increasing the potency, specificity, bioavailability, and metabolic stability in M. tuberculosis itself.

Grant: 1R21AI055773-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: GILL, RYAN PHD
Title: Using Genomics to Identify Antibiotic Sensitivity Genes
Institution: UNIVERSITY OF COLORADO AT BOULDER BOULDER, CO
Project Period: 2003/05/01-2005/04/30

DESCRIPTION (provided by applicant): Antibiotic resistance is a growing problem that affects the treatment of almost all infectious diseases. The cost of treating antibiotic resistant infections is close to \$30 billion/year in the United States alone. The identification of genes and mechanisms underlying resistant phenotypes is a focus of current drug discovery efforts wherein the goal is to develop combination therapies that target a process essential for bacterial survival and simultaneously render resistance mechanisms ineffective. On the way to this goal we believe it is necessary to understand the mechanisms by which resistant pathogens can once again be made sensitive to antibiotics and then exploit these mechanisms in the development of combination therapy. The objective of this study is to develop a new genomics based approach for the efficient identification of genes whose overexpression restores sensitivity to antibiotics in resistant pathogens and apply that approach. In the case of aminoglycoside resistant *Pseudomonas aeruginosa*, a significant cause of morbidity and mortality, particularly in cystic fibrosis patients, in addition to studying naturally occurring resistant clinical isolates of *P. aeruginosa*, two clinically relevant mechanisms of resistance will be engineered into PAO1 a sensitive strain of *P. aeruginosa*. This will be done with a plasmid either expressing the enzyme 6"-N-aminoglycoside acetyltransferase⁷, which modifies aminoglycosides or expressing the *phoP* gene, which reduces bacterial permeability to aminoglycosides. Transforming these resistant strains with a genomic library will then allow for a DNA microarray based method to identify clones expressing genomic inserts which are absent in a bacterial population grown in the presence of aminoglycosides. It is these inserts which presumably restore a sensitive phenotype to these resistant strains, causing bacterial death and an absence of these inserts in the population. Confirmatory and then mechanistic studies will be carried out to further elucidate the mechanisms behind the sensitivity phenotypes discovered.

Grant: 1R21AI055774-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: O'TOOLE, GEORGE A BS
Title: Biofilm Antibiotic Resistance in Staphylococcus
Institution: DARTMOUTH COLLEGE HANOVER, NH
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): Biofilms are complex bacterial communities attached to a surface. The most widely recognized property of biofilm bacteria is their increased resistance to antimicrobial agents. The recalcitrance of biofilm-related infections to conventional antibiotic therapy has a profound impact on the medical industry and human health. Although this problem has been studied in numerous different model systems, little is known about the molecular mechanisms that confer antibiotic resistance to biofilm cells. Recent work suggests that a genetic program controls biofilm formation and we have genetic evidence that indicates that the development of antibiotic resistance in biofilms is similarly regulated. Furthermore, so-called "small colony variants" or SCV have been proposed to play a role in biofilm-mediated resistance in *P. aeruginosa*. The role of SCV in *S. aureus* biofilm resistance has not yet been investigated. The central hypothesis of this application is that antimicrobial resistance of biofilm-grown cells requires specific genetic elements. We propose to identify genetic elements required for the development of biofilm antibiotic resistance by *Staphylococcus aureus* using techniques developed in our previous studies in *Pseudomonas aeruginosa*. The identification of genes required for biofilm antibiotic resistance may provide new targets for anti-biofilm therapies and increase our understanding of biofilm antibiotic resistance. The Specific Aims of this application are: Specific Aim 1. Identify genetic elements required for biofilm antibiotic resistance in *S. aureus*. Specific Aim 2. Characterize mutants defective in biofilm antibiotic resistance. Specific Aim 3. Determine the role of small colony variants (SCV) in biofilm antibiotic resistance. The studies proposed here explore a poorly characterized aspect of microbial resistance that is elaborated when microbes grow in a biofilm. No genetic elements contributing to biofilm resistance have been identified in *S. aureus*. We propose a genetic screen (already validated in *P. aeruginosa*) with the goal of identifying genes involved in this process. Future studies will uncover the mechanisms of resistance mediated by the genes identified as a result of this work. We will also perform studies to determine whether SCV, thought to be generated in chronic bacterial infections in vivo: i) play a role in biofilm resistance and ii) utilize the same genetic pathways as biofilm-grown cells to resist the action of antibiotics.

Grant: 1R21AI055778-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: JANDA, KIM D
Title: Antibody and peptide inhibitors of quorum sensing
Institution: SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA
Project Period: 2003/03/15-2005/02/28

DESCRIPTION (provided by applicant): *Staphylococcus aureus* is a leading cause of diseases ranging from skin infections and food poisoning to life-threatening nosocomial infections. Increasing resistance of *S. aureus* isolates to glycopeptide antibiotics, most prominently vancomycin, is a major concern in today's intensive care units (ICUs). Many of the genes controlling the virulence of *S. aureus*, such as exotoxins, are regulated through a quorum sensing mechanism and may contribute to an antibiotic-resistant phenotype. The quorum sensing signaling molecules used by *S. aureus* are small cyclic peptides, also called autoinducing peptides (AIPs), whose primary structures, i.e. their amino acid sequences, vary among different strains of *S. aureus*. They are actively secreted through a transporter protein, AgrB, and bind in auto- and paracrine fashion to their cognate receptor, AgrC. This receptor is part of a classical two-component signal system that includes AgrA as the response regulator. The signal is transduced from AgrC to AgrA, triggering the expression of a particular set of virulence genes. It has been shown that by blocking this signaling pathway, the expression of virulence factors can be inhibited and *S. aureus* pathogenicity can be attenuated. Our proposed work includes the following aims: (1) synthesis of quorum sensing peptides and analogs from all 4 AIP subgroups of *S. aureus*; (2) selection of fully human antibody fragments against these peptides through the use of phage display technology; (3) selection and identification of human antibodies and peptides that bind to the extracellular domains of AgrB or AgrC from AIP-subgroup I *S. aureus*; (4) evaluation and characterization of the selected peptides and antibodies for their ability to successfully block the agr-based signaling cascade of AIP-subgroup I *S. aureus*. The work proposed herein represents a novel strategy to combat antibiotic resistance in *S. aureus*.

Grant: 1R21AI055781-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: JANDA, KIM D
Title: Generating antibodies against N-Acyl homoserine lactones
Institution: SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): The discovery that Gram-negative bacteria employ small molecules, namely N-acyl homoserine lactones (AHLs), to globally regulate the production of secondary metabolites and control the expression of virulence determinants has provided a new potential target for anti-infective therapy. Genes regulated through this pathway include exotoxins and other pathogenicity factors, as well as genes for bacterial self-defense such as biofilm formation, which contribute to antibiotic resistance. Interference with this pathway, either by sequestration of the AHLs or cleavage of the lactone bond, has been shown to attenuate pathogenic bacteria and render them harmless. Throughout the realm of Gram-negative bacteria these AHL-signaling molecules differ only in their acyl moiety, leaving the homoserine lactone as the core structure. This feature makes AHLs an attractive target for anti-microbial antibody therapy. Specifically, the aims of this application are (1) the chemical synthesis of AHL-based analogues, including: a) a phosphonate transition state analogue (TSA) of lactone hydrolysis, b) native lactone structures that differ only in the acyl chain substitution pattern; (2) generation of monoclonal antibodies (mAbs) against these AHL-based haptens, with the TSA being designed such that catalytic antibodies will be elicited that are capable of hydrolyzing the lactone bond and thereby inactivate the signaling molecule; (3) the isolation of fully human antibody fragments that bind AHL; (4) characterization and evaluation of these antibodies for their ability to sequester or inactivate AHL molecules and so inhibit the quorum sensing signaling pathway. The work proposed herein represents a novel strategy to combat antibiotic-resistant Gram-negative bacteria using immunotherapy.

Grant: 1R21AI055800-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: VICTOR, THOMAS C PHD
Title: INH resistance in Beijing/W isolates of M tuberculosis
Institution: UNIVERSITY OF STELLENBOSCH STELLENBOSCH,
Project Period: 2003/06/01-2005/05/31

DESCRIPTION (provided by applicant): The communities in the Western Cape of South Africa have one of the highest incidences of tuberculosis in the world with reported outbreaks of drug resistance. This study is based on the observation that the prevalence of INH mono-resistance in local Beijing/W-like drug resistant isolates is high and no gene mutations associated with INH resistance in known genes have yet been identified. It is likely that INH mono-resistance in this family of strains is due to interaction of a combination of bacterial and host risk factors. The overall aim in this pilot study is to identify risk factors associated with Isoniazid resistance which may be pathogen and/or host specific and which may lead to acquisition of MDR-TB in Beijing/W-like strains after controlling for compliance. Two groups of patients will be identified from 72 clinics in the Western Cape. Group 1 will consist of patients infected with drug resistant Beijing/W isolates and group 2 will be patients infected with drug susceptible Beijing/W isolates. The isolates from both groups will then be further phenotypically and genotypically characterized to (i) confirm that the isolates truly belong to the Beijing Family of strains, (ii) identify different clusters as a measure of the propensity of different W-like cluster sizes to develop a drug resistant phenotype and to transmit, (iii) identify Beijing/W isolates with and without INH associated gene mutations as a function of the MIC for INH so that these isolates can be used to identify possible INH induced alterations by protein expression profiling in 2D gels. All patients from groups one and two and a control group without Tuberculosis disease will be retrospectively recruited to give blood to determine the N acetyltransferase allele status, as a measure to metabolize INH in vivo during treatment. In addition, socio-economic, clinical and demographic data will be collected from all patients infected with Beijing/W strains to control for possible confounding. The reference database and well-characterized Beijing/W isolates from this study will lay the foundation to further study the highly prevalent Beijing/W family of strains in local communities. It will also allow comparative analysis to other local outbreak drug resistant strains and will help unravel the mechanisms that lead to the development and spread of drug resistance in local communities.

Grant: 1R21AI055818-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: HOTCHKISS, JOHN R MD
Title: Dynamics of Peritoneal Dialysis Associated Peritonitis
Institution: HEALTH PARTNERS RESEARCH MINNEAPOLIS, MN
FOUNDATION
Project Period: 2003/07/01-2004/06/30

DESCRIPTION (provided by applicant): This multidisciplinary pilot application focuses on interactions between dynamics of bacterial growth and optimal bacterial clearance in the setting of peritoneal dialysis associated peritonitis (PDAP). In the proposed work, we will 1) use in vitro models to define the dynamics and nutrient dependence of fluid phase bacterial growth and antibiotic-induced bacterial killing in peritoneal dialysate (PDF); 2) develop and validate mathematical models of bacterial growth and killing in PDF as functions of time, growth rate, and nutrient availability; 3) Develop mathematical models of bacterial clearance (antibiotic and non-antibiotic related) during peritoneal dialysis. Dynamics guided optimization of bacterial clearance during peritonitis will allow reduction in the antibiotic exposure "burden" for these dialysis patients and their bacterial flora. In addition, this work may provide preliminary data regarding bacterial growth dynamics and antibiotic susceptibility in other "stressful" environments having single or multiple nutrient deficiencies. In peritoneal dialysis, the abdominal cavity is intermittently filled with a fluid (PDF) that is allowed to dwell in the peritoneal cavity. During this dwell time, waste products, excess salts, and water diffuse into the PDF. The PDF is subsequently drained; carrying with it the accumulated waste products and water, and the peritoneal cavity is refilled. Bacteria in fresh PDF represent a starved population, with consequent and profound suppression of bacterial division; starved *S. aureus* may require 2-2.5 hours to resume active cell division following provision of adequate nutrients. As the dwell times commonly used in peritoneal dialysis are in the 4-6 hour range, this delay in growth onset could be clinically significant, both because it may offer a means to augment non-antibiotic-mediated bacterial clearance ("flushing out" the peritoneal cavity), and because slowing or halting bacterial growth may decrease the antibacterial activity of commonly used antibiotics. Moreover, the significant impairment of host peritoneal defenses due to the non-physiologic milieu in PD fluid renders non-host factors, such as antibiotic efficacy and dialysis prescription, more important in clearing peritoneal infection. Prolonged courses of antibiotic therapy required by compromised host defenses may render the dialysis population an effective "incubator" for drug resistant microorganisms.

Grant: 1R21AI055821-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: MILLER, MICHAEL H MD
Title: Role of Quinolone Efflux Pumps in *S. pneumoniae* Resistance
Institution: ALBANY MEDICAL COLLEGE OF UNION ALBANY, NY
UNIV
Project Period: 2003/07/01-2005/06/30

DESCRIPTION (provided by applicant): Relevance to R21 RFA: Our application will characterize PmrA, the quinolone efflux pump of *S. pneumoniae* that is overexpressed in response to hydrophilic quinolones. We believe that this overexpression leads to an unnecessarily high rate of resistance. This application is novel since it focuses on the role of efflux pumps in predisposing to subsequent mutations in topoisomerase genes rather than primary mutations in the topoisomerase genes themselves. We believe that mutations in *S. pneumoniae* causing constitutive overexpression of its efflux pump [PmrA], as well induction causing efflux pump upregulation in WT cells, predispose to topoisomerase mutations. Notably, the chemicals that cause quinolone pump induction in other bacteria include quinolones themselves. Global regulation in response to environmental conditions may also upregulate PmrA leading to resistance. Specific Aim #1 will characterize mutational PmrA upregulation as well as pump induction in WT cells and the effects this has on quinolone susceptibility. Preliminary studies will identify chemicals that are pump substrates, which are also potential inducers, by comparing their MICs in the presence and absence of reserpine. To survey these potential inducers we will use a pmrAlacZ reporter in a α -galactosidase negative strain of *S. pneumoniae*, mRNA and protein will be measured in the presence of inducers in WT strains and in strains that do not express pmrA as well as mutants with constitutive expression by Real Time PCR and Western blots. Specific Aim #2 will characterize the efflux kinetics of quinolones with different hydrophilicities using everted membrane vesicles of *E. coli* that express the PmrA efflux pump of *S. pneumoniae*. In these studies we will use radiolabeled quinolones, uptake measured by membrane filtrations, the Vmax and Km will be determined. Long term objectives: We expect that the new information we provide will lead to more rational approaches to quinolone therapy considering ways to prevent efflux pump mutant selection or upregulation in WT cells minimizing subsequent topoisomerase mutant selection. Using everted vesicles in conjunction with kinetic data from other subcellular systems describing kinetic parameters describing uptake and topoisomerase interactions, ultimately will provide a model to quantify the relative roles each has on resistance as well as develop a novel, mechanism related screen that may be useful in developing new quinolones with enhanced activity against resistant isolates.

Grant: 1R21AI055822-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: CUNNINGHAM, PHILIP R PHD
Title: Technology for new tuberculosis anti-infectives
Institution: WAYNE STATE UNIVERSITY DETROIT, MI
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): A genetic system was developed in *Escherichia coli* that uses combinatorial genetics to identify mutations in ribosomal RNA (rRNA) drug targets that might lead to antibiotic resistance. Recently, the 16S RNA from *Mycobacterium tuberculosis* was substituted for *E. coli* 16S RNA in this system but the construct produced inactive ribosomes when expressed in *E. coli*. Hybrid 16S rRNAs containing the 5' and central domains from *E. coli* and the 3' major and minor domains from *M. tuberculosis*, however, produce active ribosomes in *E. coli*. This suggests that nucleotide differences in the 5' and/or central domain of *M. tuberculosis* 16S RNA are responsible for loss of function when expressed in *E. coli*. Absence of function in 30S subunits composed entirely of *M. tuberculosis* 16S rRNA is probably due to the inability of a nucleotide(s) in *M. tuberculosis* 16S RNA to interact with an *E. coli* 30S ligand(s). The goal of this project is to develop genetic technology for the isolation of new anti-infectives that address the issue of drug resistance in *M. tuberculosis*. Two aims are proposed: (1) The nucleotides in *M. tuberculosis* rRNA responsible for loss of function in *E. coli* will be identified and (2) the *M. tuberculosis* 30S ligand(s) required for expression of *M. tuberculosis* 16S RNA in *E. coli* will be identified and cloned. Co-expression of *M. tuberculosis* 16S RNA and the ligands should produce functional ribosomes containing *M. tuberculosis* 16S RNA in *E. coli*. Drug resistance in *M. tuberculosis* is due primarily to chromosomal mutations in the drug targets. Multi-drug resistance appears to occur through sequential accumulation of such mutations. For target-site mutations to be clinically significant, the mutated target must retain most of its biological activity since loss of function decreases the fitness and virulence of the pathogen. This is especially so for functional regions of rRNA, which are critical for protein synthesis. Successful completion of this project will provide a technology to develop novel anti-infectives that recognize all possible functional forms of the target, even if not yet found in nature, and are therefore unlikely to be susceptible to the development of resistance through target modification. Once developed, this technology will allow the use of rRNA genes from other microbial pathogens in designing new anti-infectives.

Grant: 1R21AI055825-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: LIPSITCH, MARC PHD
Title: Epidemiologic Methods: Resistant Nosocomial Infections
Institution: HARVARD UNIVERSITY (SCH OF PUBLIC BOSTON, MA
HLTH)
Project Period: 2003/04/15-2005/03/31

DESCRIPTION (provided by applicant): The growth of antimicrobial-resistant nosocomial infections (ARNI) necessitates the identification and widespread implementation of effective interventions to reduce their incidence, such as changes in prescribing and improvements in infection control. Candidate interventions are often identified by observational studies of modifiable risk factors for ARNI; candidates are then evaluated in clinical trials. Existing methods for both observational studies and clinical trials assume that patient outcomes are independent of each other. This is not true for ARNI, because pathogens are transmissible, so infection of one host may make others more likely to be infected; similarly, use of antibiotics by others in the hospital can increase an individual's risk of ARNI, even if s/he has not received the drug. We have shown that nonindependence is common in ARNI data, obscures the mechanistic effects of antimicrobial use on the incidence of ARNI, and can lead to false results (negative or positive) when interventions are assessed; thus, there is an emerging consensus on the inadequacy of many existing studies and the need for better methods. We will develop and test methods for observational studies and clinical trials that account for nonindependence of patients. For observational studies, we will use data from the University of Utah (UU) to assess simultaneously the effects of individual antibiotic use and total hospital-wide use on risk of ARNI. For clinical trials, we will develop three methods for evaluating interventions while accounting for nonindependence. We will test these methods on real data from UU and the CDC/Emory ICARE project, and on simulated data, for their fit to data, ability to detect effective interventions, and ability to avoid false positive detection of intervention effects that are not real. Methods will include an auto regressive negative binomial model, which is easily implemented in standard software, and more sophisticated approaches, such as hidden Markov models. We will identify methods that perform well on data and will reliably determine the effectiveness of interventions. Dissemination of the results of these studies via peer-reviewed publications, free distribution of software, didactic seminars and future work with specific collaborators will aid in the reliable identification of candidate interventions and trustworthy ways to assess whether these interventions work. This will in turn lead to better practices to reduce the incidence of ARNI.

Grant: 1R21AI055883-01
Program Director: TAYLOR, KATHERINE A.
Principal Investigator: BRIGGS, JAMES M PHD
Title: Dynamic Drug Design Targeting Botulinum Neurotoxins
Institution: UNIVERSITY OF HOUSTON HOUSTON, TX
Project Period: 2003/08/15-2005/07/31

DESCRIPTION (provided by applicant): Botulinum neurotoxins (BoNTs) are a dangerous bioterrorism threat due to their extreme potency and lethality, as well as their ease of production and transport. If untreated, poisoning by the BoNTs can progress to flaccid paralysis and death due to respiratory failure. However, timely post-exposure intervention can limit the effects of the circulating toxin. Our overall, long-term research objective is to generate a novel class of therapeutics that can be administered to individuals who have been poisoned by BoNT. Each BoNT is composed of a catalytic light chain whose entry into neurons is mediated by the heavy chain. Our strategy is based on the model that botulism-related flaccid paralysis is a downstream consequence of the zinc-dependent endopeptidase activity elaborated by the BoNT light chain. One of the most powerful approaches to inactivate the endopeptidase function of the BoNT light chains is rational design of inhibitors targeting the active site. To achieve this, we will combine computational and experimental approaches to develop lead inhibitor templates. In Specific Aim 1, we will use a powerful computational approach called dynamic pharmacophore modeling to identify computational leads to block the endopeptidase activities of the BoNTs. In this approach, the conformational flexibility of the protein and active site are taken into account through molecular dynamics simulations and the generation of a consensus, or dynamic, pharmacophore model using an ensemble of molecular dynamics-generated protein conformations. The dynamic pharmacophore model is then used to search databases of commercially available small molecules to generate computational lead compounds. In Specific Aim 2, we will test each computational lead for inhibitory activity using enzyme assays and in vitro cellular assays. A milestone of this work will be the identification of one or more lead inhibitor templates that block the action of wild type toxin using in vitro model systems. The results from this research will establish the groundwork and justification for future development and in vivo testing of these novel inhibitors using established animal models.

Grant: 1R21AI055900-01
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: STARNBACH, MICHAEL N. PHD
Title: Single Cell Expression Profiling of Chlamydia Genes
Institution: HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA
Project Period: 2003/07/15-2005/06/30

DESCRIPTION (provided by applicant): Chlamydia trachomatis is an intracellular pathogen that is responsible for significant human morbidity throughout the world. Under the funded grant linked to this proposal (5 R01 AI039558-07) we have been identifying and testing a number of T cell antigens for their role in protective immunity to C. trachomatis. Through the work described in this Exploratory/Developmental application, we propose to use the published genome sequence and fluorescence in situ hybridization (FISH) technology to identify candidate C. trachomatis T cell antigens based on their expression at the relevant time in the developmental cycle and their expression in the relevant tissues. Little is known about gene expression in these organisms during their developmental cycle because of the difficulty in synchronizing Chlamydia infections. The use of FISH technology circumvents the problem of asynchronous infections because we will be able to analyze the expression of Chlamydia genes at the level of individual bacteria. In the first Specific Aim, we propose to use FISH to explore the developmental cycle of C. trachomatis in cultured cells. Using FISH, we should be able to detect induction or repression of genes that were previously undetectable using other techniques. After optimizing probe design and hybridization conditions, we will use FISH to simultaneously monitor the expression of multiple genes within an organism using probes tagged with different fluorescent dyes. We will then compile a database containing the relative levels of expression of many C. trachomatis genes at various times in the developmental cycle. This information will be applied to the rational identification of candidate T cell antigens based on their expression early in intracellular development, when a T cell response would be most effective. The database will also aid in deciphering the pathways and regulatory networks present at each developmental stage. In the second Specific Aim, we propose to use FISH to study Chlamydia gene expression in genital tissues during murine infection. In particular, we will look at the temporal and spatial differences in Chlamydia gene expression in these tissues. We also propose to analyze changes in Chlamydia gene expression under various host immunological or chemotherapeutic pressures. These data will also allow for the identification of candidate antigens expressed in appropriate tissue types at times when T cell recognition would be most effective.

Grant: 1R21AI055907-01
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: SIMECKA, JERRY W PHD MICROBIOLOGY, OTB
Title: Modulation of cytokine networks in mycoplasma pneumonia
Institution: UNIVERSITY OF NORTH TEXAS HLTH SCI FORT WORTH, TX
CTR
Project Period: 2003/07/01-2005/06/30

DESCRIPTION (provided by applicant): The broad, long-term objective of the parent grant (5 R01 AI42075-04) and this project is to determine the immune and inflammatory responses that have an impact on the pathogenesis of and protection from mycoplasma respiratory disease. In the parent grant, the hypothesis is that T helper cells play a central role in determining the balance between helpful and harmful immunologic responses in murine mycoplasma respiratory disease. Furthermore, this balance is mediated by T cell subsets whose activity is modulated by regulatory cytokines, such as IL-4 and IFN-gamma. In fact, Th cells are proinflammatory while CD8+ T cells were found to unexpectedly dampen the inflammatory disease due to mycoplasma infection. In addition, the results demonstrate that IFN-gamma plays a novel but an important regulatory role in innate immunity against mycoplasma. These results indicate that the immune mechanisms involved in this disease are complex and require a broader understanding of the regulatory cytokines produced during disease pathogenesis and their regulation by T cells and cytokines. Thus, the current studies are proposed to establish whether a broader, more exploratory approach to analyze production of cytokines can facilitate our understanding their modulation and their potential role in mycoplasma respiratory disease. The Specific Aims address the following questions: 1) What cytokines are produced in lungs of normal or IFN-gamma deficient mice at different stages of mycoplasma disease?; and 2) What is the impact of CD4+ and CD8+ T cells on pulmonary cytokine production? The methods to be used in this exploratory approach are to first monitor cytokine mRNA production using array technology, and those cytokine mRNAs, shown modulated using arrays, will be further analyzed for their levels of expression and for experimental variations using real-time RT-PCR analysis. Furthermore, cytokine protein arrays will be developed to assess their production in a microarray format; once established tissue homogenates and cell culture supernatants to do this, we will develop assays using pre-existing antibodies to capture and detect cytokine protein in an array format. Thus, by analysis of a broad number of cytokines, clusters of cytokines with similar and/or contrasting regulatory properties should be identified that are associated with disease progression for further functional analysis.

Grant: 1R21AI055929-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: KHAN, SALEEM A. PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: PcrA Helicase and Replication of Drug Resistance Plasmid
Institution: UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PA
PITTSBURGH
Project Period: 2003/04/15-2005/03/31

DESCRIPTION (provided by applicant): Plasmids that replicate by a rolling-circle (RC) mechanism are ubiquitous in Gram-positive bacteria and a vast majority of over 200 rolling-circle replicating (RCR) plasmids belong to four major families. Many RCR plasmids carry antibiotic resistance genes and evidence suggests that horizontal transfer of RCR plasmids is quite common. Many RCR plasmids also contain genes that are involved in plasmid mobilization and transfer. While some RCR plasmids are able to replicate stably only in their native hosts, many have a broad host range. RC replication involves synthesis of the leading strand that requires the PcrA helicase, single strand DNA binding protein and DNA polymerase III. Lagging strand replication requires primer RNA synthesis by the host RNA polymerase, and subsequently replication is carried out by DNA Pol I and DNA Pol III. PcrA helicase is required for plasmid RC replication as well as survival of Gram-positive organisms. The goal of this R21 application is to test the hypothesis that interaction between plasmid initiator proteins and the PcrA helicases is critical for efficient plasmid replication and may determine narrow versus broad host range replication of RCR plasmids. No studies have been performed dealing with this issue and the exploratory/development nature of the current application is consistent with the R21 format. We plan to utilize a few plasmids with a relatively narrow host range and a few with broad host range in our study. We will study the replication of these plasmids in *S. aureus*, *B. subtilis*, *B. cereus* and *S. pneumoniae*. RCR plasmids defective in replication in a particular host will be complemented by a cloned copy of their cognate *pcrA* gene. We will purify the PcrA helicases from the above Gram-positive organisms and study their interactions with the plasmid initiator proteins. We will also study the in vitro replication of the above plasmids in cell free extracts made from *S. aureus* in the presence and absence of the various PcrA helicases. These studies should reveal whether PcrA is critical in determining the efficient replication and host range of RCR plasmids. These studies may facilitate the development of antimicrobial drugs targeting the PcrA helicase and/or the initiators of RCR plasmids.

Grant: 1R21AI055946-01
Program Director: TAYLOR, KATHERINE A.
Principal Investigator: ZENG, MINGTAO PHD
Title: New genetic vaccine to protect against botulism
Institution: UNIVERSITY OF ROCHESTER ROCHESTER, NY
Project Period: 2003/08/01-2005/07/31

DESCRIPTION (provided by applicant): Botulism is a severe neuromuscular disease caused by one of seven botulinum neurotoxins (BoNTs), produced by the anaerobic, spore-forming bacterium *Clostridium botulinum*. These protein neurotoxins are the most potent toxins known to man. There are BoNT toxoid vaccines available currently as Investigational New Drugs. However, due to the numerous shortcomings associated with the toxoid vaccines (i.e., dangerous to produce, high cost of manufacturing, high reactogenicity), there is an urgent need to develop new generation vaccines for the prevention of botulism. The goals of this research are to develop a new botulism vaccine using the carboxyl-terminal 50 kDa C-fragments (Hc) of the heavy chains in BoNTs as antigens and to study the delivery of the vaccine utilizing a replication-defective adenoviral vector via the intranasal and transcutaneous routes. These non-invasive vaccine delivery methods will undoubtedly enhance the compliance of a vaccination program, which is especially critical in response to a potential bioterrorist attack using BoNTs. After construction of replication-defective adenoviral vectors encoding the immunogenic C-fragments of the heavy chains in BoNTs, vaccination protocols in mice comparing the intranasal and transcutaneous delivery modes with the subcutaneous injection of the currently available pentavalent botulinum toxoid vaccine (PBT) will be studied. The specific aims of this project are: Specific Aim #1: To construct replication-defective adenoviral vectors encoding the C-fragments of the heavy chains in BoNTs. Specific Aim #2: To study the mucosal and systemic immunity elicited by the vectored vaccine developed in aim #1 through intranasal and transcutaneous immunization in a mouse model.

Grant: 1R21AI055955-01
Program Director: VAN DE VERG, LILLIAN L.
Principal Investigator: SAPER, MARK A PHD
Title: Structures of a Conserved Type III Effector Domain
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2003/07/01-2005/06/30

DESCRIPTION (provided by applicant): Many animal and plant pathogenic bacteria, including at least seven on the NIAID priority pathogen list, deliver virulence proteins (effectors) directly into host cells through type III secretion (TTS) systems. There they disrupt cell signaling to manipulate the cell for the bacteria's advantage. This proposal investigates the structural determinants of how specific effector proteins are recognized by the TTS apparatus for subsequent secretion and translocation. *Salmonella typhimurium* is a class B pathogen that causes severe diarrhea in people and livestock. For some effectors, a small chaperone protein binds to a non-conserved region near the amino-terminus of the effector to ensure efficient translocation. Interestingly, in *S. typhimurium*, a set of nine effectors have homologous amino-terminal domains (about 145 residues, termed 'WEKIF' domains) but are unrelated elsewhere. These domains are required and sufficient for translocation; no secretion chaperones have been identified and they may not be required. The conserved nature of the domain suggests that it also may localize the effector to specific compartments or proteins in the host cell. The long-term goal of the research is to define the structural basis for the protein:protein interactions involving this domain. This is the first step for discovering potential targets for antimicrobial development. Comparative structural studies of the individual WEKIF domains are proposed here. Aim 1 of the proposed research will improve existing crystals of the WEKIF domain of the SspH1 effector, and begin crystallographic structure determination. Aim 2 proposes to express and purify four other WEKIF domains, SirP, SifA, SseI, and SseJ, and screen for crystallization conditions. Following up on a related, but positive result, Aim 3 will screen the full-length SspH1, containing the leucine-rich repeat effector domains, for crystals.

Grant: 1R21AI055964-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: ENNIS, DON G PHD
Title: Attenuation of Brucella Using Dominant Repair Mutants
Institution: UNIVERSITY OF LOUISIANA AT LAFAYETTE LAFAYETTE, LA
Project Period: 2003/08/01-2005/07/31

DESCRIPTION (provided by applicant): The principal goal of this proposal is to develop new genetic tools that permit the rapid construction of repair-defective pathogenic bacteria. Our approach is called TAISR (for Trans-Acting Inhibition of SOS Repair) which introduces vectors carrying dominant mutant genes that "poison" SOS repair activities. A requirement for DNA repair functions in pathogenesis has been documented for some intracellular pathogens. For example, repair-defective mutants of Salmonella were completely attenuated in mice; this loss of virulence was traced to their inability to repair DNA damage within macrophages. A number of pathogens not only survive but even flourish within professional phagocytes; these include some Brucellaceae, Campylobacteriaceae, Edwardsiellaceae, Francisellaceae, Listeriaceae, Mycobacteriaceae and Yersiniaceae. We are investigating the role of repair in pathogenesis by Brucella abortus an intracellular pathogen that causes undulant fever in humans and induces abortions in animals. We found that some B. abortus repair mutants, constructed by gene-disruptions, can decrease its ability to survive and grow in macrophages by 3,000-fold. Preliminary studies using a *lexA*-based TAISR system revealed partial poisoning of B. abortus SOS repair and a 100-fold greater killing in macrophages. We are proposing to improve TAISR by developing more effective dominant genes as well as use mutant combinations to inactivate repair in B. abortus. Attenuated mutants or mutant combinations generated by TAISR will provide insights into the design of live vaccines against Brucella. These genetic tools will have broad applications; they may be used to construct attenuated mutants in variety of bacterial pathogens. We are proposing the following specific aims for developing convenient genetic tools to construct attenuated mutants of Brucella. 1) Characterization of repair-defective B. abortus carrying dominant mutants from E. coli (*lexA*, *recA* and *ruvB*) as well as mutant combinations. 2.) Functional and molecular characterization of cloned copies of the *lexA*, *recA* and *ruvB* homologs from B. abortus. 3) Site-directed mutagenesis to construct dominant mutants from these three B. abortus repair genes. 4) Characterization of B. abortus strains expressing dominant *lexA*, *recA* and *ruvB* mutants from B. abortus.

Grant: 1R21AI055966-01
Program Director: SCHMITT, CLARE K.
Principal Investigator: DWINELL, MICHAEL B BA
Title: Chemokines in host defense to Campylobacter jejuni
Institution: MEDICAL COLLEGE OF WISCONSIN MILWAUKEE, WI
Project Period: 2003/09/30-2005/08/31

DESCRIPTION (provided by applicant): This R21 research proposal, submitted in response to "Biodefense and Emerging Infectious Diseases Research Opportunities", NOT-AI-02-023, has two specific aims designed to increase our understanding of the pathogenesis of Campylobacter jejuni enterocolitis. The intestinal epithelium comprises a dynamic physical barrier that maintains an active repertoire of innate host defense responses to limit entry of clinically significant food- and water-borne pathogens. These mechanisms include the regulated production of chemokines to coordinate the appropriate innate and adaptive immune effector response. C. jejuni is a leading cause of bacterial diarrheal disease in the world. However, while relatively little is known of the pathophysiologic mechanisms employed to infect the human intestinal tract and elicit disease, interaction at the intestinal epithelium is the most common pathogenic feature of infection. The overall objective of this research proposal is to obtain novel information on the mechanisms of pathogenesis to C. jejuni enterocolitis and will, as an important first step, focus on the coordinated production of chemokines by the cells of the intestinal epithelium as a significant host defense mechanism. Studies in Aim 1 will test the hypothesis that C. jejuni infection of human intestinal epithelial cells stimulates production of chemokines for neutrophils, dendritic cells and T lymphocytes, effector cells that we postulate act in concert to limit C. jejuni entry in vivo. A culture model intestinal epithelium will be infected with C. jejuni and the signaling mechanisms regulating epithelial chemokine production assessed. To define bacterial pathogenicity, studies in Aim 2 will utilize C. jejuni mutants to test the hypothesis that specific Campylobacter virulence factors induce host epithelial cell chemokine expression. Induction of epithelial chemokine expression will be tested in C. jejuni flagella mutants, as well as mutants selected from candidates revealed from a promoter trap-based approach to define novel virulence factors. Together, these studies will provide new insights into the cellular signaling mechanisms and bacterial gene products regulating intestinal epithelial chemokine production as a central host defense function to C. jejuni. Understanding the cellular and biochemical mechanisms of intestinal epithelial host defense to human C. jejuni infection are central to the development of preventative therapeutic strategies to modulate host-pathogen interactions to favor the host.

Grant: 1R21AI055968-01
Program Director: BEANAN, MAUREEN J.
Principal Investigator: JOHN, MANOHAR PHD
Title: Anthrax spore-surface antigens for biosensor development
Institution: MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA
Project Period: 2003/08/01-2005/07/31

DESCRIPTION (provided by investigator): The accurate detection of an aerosol of weaponized anthrax spores, using antibody-based biosensor technology, is centrally dependent upon antibodies of singular specificity for anthrax spore-surface antigens. As an initial step toward this objective, we propose the identification of a pool of genes encoding such antigens, using a combination of genetic (RT-PCR), and immunological (immunoscreening) techniques. Genes isolated using this combination strategy, will then be identified by a BLAST search against sequences contained in the National Center for Biotechnology Information (NCBI) non-redundant database, and against the *B. anthracis* genome sequence available at The Institute for Genomic Research website (www.TIGR.org). Owing to time and budgetary constraints, in this study, only a few (one or two) of the identified genes will be selected for polyclonal antibody development, which will be generated via genetic immunization (GI). Selection will be based on whether the identified genes contain the consensus promoter sequence for the binding of sigma factor, (σ), which directs transcription of several genes during late sporulation, and also whether such genes are unique to *B. anthracis* as determined by BLAST and Southern blotting experiments. A rational approach will be used in the selection of DNA sequences for GI in order to prevent or minimize cross reactivity of the polyclonal antibodies with spores of close relatives. To accomplish this, protein sequences predicted by genes that meet the above criteria, will be extensively analyzed using protein analytical tools. Regions of such genes that encode antigenic domains not part of motifs, patterns, signatures, active enzyme sites or other sequences, common to sequenced members of the genus *Bacillus*, will then be amplified, cloned into a suitable plasmid DNA (pDNA) vaccine vector, and used to elicit polyclonal antibodies via GI. Gene sequences encoding domains, which elicit polyclonal antibodies that strongly react with anthrax spores in an intact spore-ELISA, and exhibit little or no cross-reactivity with those of close relatives, will be used to generate monoclonal antibodies (MAbs) via GI. Following evaluation of MAbs in the spore-ELISA, highly specific polyclonal and MAbs will be evaluated further in a micro electrical and mechanical (MEMS) sensor device in the future, with the long-term goal of developing a portable, antibody-based sensor for the detection of aerosolized anthrax spores.

Grant: 1R21AI056042-01
Program Director: SCHMITT, CLARE K.
Principal Investigator: YOUNG, GLENN M BS
Title: YSA locus of high-virulent *Yersinia enterocolitica*
Institution: UNIVERSITY OF CALIFORNIA DAVIS DAVIS, CA
Project Period: 2003/09/01-2005/08/31

DESCRIPTION (provided by applicant): Pathogenic *Yersiniae* are excellent models for the study of bacterial-host interactions at the molecular level. Important paradigms -- such as contact-dependent Type III secretion systems that deliver bacterial proteins to the cytoplasm of host cells -- have emerged from the study of three pathogenic species: *Y. enterocolitica* and *Y. pseudotuberculosis*, which are enteropathogens causing a wide range of gastrointestinal symptoms; and *Y. pestis*, which is the etiological agent of plague. Type III secretion systems (TTSS) were first described in *Yersiniae*, and the well-characterized Ysc TTSS is found in all three pathogenic species. Our long-term goal is to understand the virulence factors that distinguish high-virulent from low-virulent strains of *Y. enterocolitica*. One such virulence factor is a second contact-dependent TTSS encoded by genes of the YSA locus, a putative pathogenicity island. This TTSS remains particularly understudied and is the focus of this proposal. The Ysa TTSS secretes a set of proteins called Ysp's and functions independently from the plasmid-encoded Ysc TTSS. Studies with both animal and cellular models of infection indicate that the Ysa TTSS is important for pathogenesis. At the cellular level macrophages are thought to play a significant role in limiting *Y. enterocolitica* infection. Notably, we have shown that cultured macrophages respond differently to infection by Ysa TTSS mutants compared to wild type *Y. enterocolitica*. The proposed research will extend these results to understand how the Ysa TTSS contributes to pathogenesis at the molecular level, and in cellular and animal models. We hypothesize that the Ysa TTSS targets one or more virulence effectors into host cells and that these in turn influence disease progression. To address this hypothesis, we have initiated three specific aims: (1) Identify virulence effectors targeted to the host cell by the Ysa TTSS; (2) Define the regulatory network that controls Ysa TTSS gene expression; and (3) Determine the role of the secreted Ysps and the Ysa TTSS in pathogenesis using animal and cell culture models of infection. This research will contribute to understanding why some pathogens have multiple TTSS systems. More specifically, it will address the question of why high-virulent *Y. enterocolitica* maintain two contact-dependent TTSS systems, each of which transports a defined set of proteins and each of which is under separate control.

Grant: 1R21AI056059-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: ESCUYER, VINCENT E PHD
Title: Virulence genes identification in *Francisella tularensis*
Institution: SOUTHERN RESEARCH INSTITUTE BIRMINGHAM, AL
Project Period: 2003/09/01-2005/08/31

DESCRIPTION (provided by applicant): *F. tularensis*, is a facultative intracellular bacterial pathogen considered as a major biological warfare agent, mainly because it is very infectious and can cause life threatening illness in humans. Without treatment, the mortality rate has been as high as 30 to 60% for the pulmonary form of tularemia, which is the most likely form in case of an act of bioterrorism. Treatment of human tularemia relies upon antibiotic therapies but relapse is frequent. Also, it is possible to engineer multiresistant strains. A live attenuated vaccine is available but confers incomplete protection, particularly if administered after exposure. Therefore, there is a crucial need for new treatments and vaccines against tularemia. However, understanding of the mechanisms of virulence in *F. tularensis* is currently too limited to address this problem efficiently. This is partly due to the lack of genetic tools, particularly a simple method of general mutagenesis. Transposable elements have become valuable mutagenic tools for genetic and molecular analysis in many different bacteria. However, no direct transposition has been observed so far in *F. tularensis*. The main goals of this proposal are as follows: first, to develop a simple and reproducible method of transposon mutagenesis in *F. tularensis*, by using a fully functional Tn5 transposition system (EZ::TN TM TransposomeTM), reconstituted in vitro. Our preliminary work has shown strong evidence for direct transposition in *F. tularensis* with this system; second, to perform transposon signature-tagged mutagenesis to isolate mutants attenuated in their virulence, in the mouse model. This pilot study should provide important informations on the genes involved in the virulence of *F. tularensis* and a better understanding of the pathogenicity of this microorganism. Some of the virulence genes will be used as potential targets for new approaches in drug design and vaccine development.

Grant: 1R21AI056061-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: CARLSON, RUSSELL W PHD
Title: Bacillus anthracis cell surface carbohydrates
Institution: UNIVERSITY OF GEORGIA ATHENS, GA
Project Period: 2003/09/30-2005/09/29

DESCRIPTION (provided by applicant): The bioterrorist threat with regard to *Bacillus anthracis* requires the development of improved vaccines, diagnostic, and therapeutic agents. Bacterial cell surface carbohydrates have been utilized for all three of these purposes, however, little is known about the *B. anthracis* cell wall carbohydrates. *B. anthracis* produces a capsule consisting of poly-g-glutamic acid, rather than a polysaccharide. However, recent work has shown that there are important carbohydrate components in both the vegetative and spore forms of *B. anthracis*. The vegetative cell wall contains a polysaccharide that is linked to peptidoglycan and serves to anchor the crystalline surface (S-layer) protein to the cell wall by binding with the SLH domain of the protein. This polysaccharide is referred to as the S-layer anchoring polysaccharide. The exosporium coat of the spore contains a major glycoprotein that is an immunodominant antigen of the spore. While the structure of the S-layer anchoring polysaccharide has not been determined, indirect data suggest that it has structural features that are specific to *B. anthracis*. There are no reports characterizing the carbohydrates of the exosporium glycoprotein. It is hypothesized that the *B. anthracis* cell wall contains carbohydrates (e.g. the S-layer anchoring polysaccharide and the exosporium glycoprotein) that are species and/or strain specific and are, therefore, candidates for the development of vaccines, diagnostic and therapeutic agents. The specific aims of this application are to (a.) determine if the S-layer anchoring polysaccharide and the exosporium carbohydrates have species specific structural features, (b.) determine if these same carbohydrates have strain specific structural features, and (c.) determine if the *B. anthracis* virulence plasmids, pX01 and pX02, encode for enzymes that structurally modify these carbohydrates. This work will be done by isolating and structurally characterizing these carbohydrates from several species of the *B. cereus* group, including *B. anthracis* var Ames, from several strains/isolates of *B. anthracis*, and from *B. anthracis* var Ames derivatives that have been differentially cured of pX01 or pX02, or of both plasmids. This work will be a collaborative effort between Dr. Russell Carlson at the Complex Carbohydrate Research Center at the University of Georgia, Dr. David Stephens at Emory University, and Dr. Conrad Quinn at the Centers for Disease Control.

Grant: 1R21AI056110-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: PETERS, WENDY PHD
Title: Chemokines and their Receptors in Anthrax Infection
Institution: J. DAVID GLADSTONE INSTITUTES SAN FRANCISCO, CA
Project Period: 2003/09/01-2005/08/31

DESCRIPTION (provided by applicant): The recent deliberate dissemination of anthrax spores has revealed significant gaps in our knowledge of anthrax pathogenesis. Macrophages play a key role in limiting anthrax infection by ingesting and eliminating the spores and vegetative bacilli. However, they also serve as a reservoir for replication and dissemination of the bacteria. We will use recently developed murine genetic models to clarify the role of the macrophages and dendritic cells (DCs) in anthrax pathogenesis. We will establish the time course of leukocyte trafficking to the lungs and draining mediastinal lymph nodes (MLNs) of mice infected with pulmonary anthrax, and we will identify the signals responsible for this recruitment. Previous work from our group has established that the monocyte chemoattractant protein (MCP) family of chemokines and their receptor, chemokine receptor 2 (CCR2), play pivotal roles in the migration of macrophages to sites of inflammation and that they are essential for host survival after infection with *Mycobacterium tuberculosis*. Unlike *M. tuberculosis*, a prominent feature of infection with *Bacillus anthracis* is the systemic effects produced by anthrax toxin acting on the macrophages. Thus, in the case of anthrax, it is unclear if impaired macrophage trafficking would be detrimental or perhaps even beneficial to the host. We will take advantage of our CCR2 mice to directly address this question in a model of pulmonary anthrax. DCs use another chemokine receptor CCR7 to traffic to draining lymph nodes (DLNs). To directly test whether CCR7, or its chemokine ligands secondary lymphoid chemokine (SLC), or Epstein-Barr ligand-1 chemokine, are involved in anthrax pathogenesis and host survival, we will take advantage of recently generated CCR7 mice. We will also attempt to establish a murine model of cutaneous anthrax infection. Progress in the understanding of cutaneous anthrax has been hampered by the lack of an adequate animal model. Once established, we will determine whether CCR2 and/or CCR7, known to regulate the trafficking of leukocytes through the skin, play a role in cutaneous anthrax. Completion of the specific aims of this proposal will establish the kinetics of leukocyte trafficking in pulmonary and cutaneous anthrax and will determine whether chemokines such as MCP-1 and SLC play important roles in phagocyte trafficking and host survival in anthrax infection. This information may provide a rationale for the use of chemokine/chemokine receptor antagonists in the treatment of anthrax.

Grant: 1R21AI056113-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: HUGHES, MOLLY A BS
Title: Studies on Macrophage Resistance to Anthrax Lethal Toxin
Institution: UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA
CHARLOTTESVILLE
Project Period: 2003/09/30-2005/09/29

DESCRIPTION (provided by applicant): Lethal toxin is a major virulence factor of *Bacillus anthracis*. Lethal toxin (LT) is comprised of two proteins, protective antigen (PA) and lethal factor (LF). Macrophages serve as the first line of defense against anthrax infection. There is a striking difference in susceptibility of mouse strains and the primary macrophages or macrophage cell lines derived from those strains to lethal, toxin-mediated effects. Certain mouse strains and their macrophages are sensitive to LT whereas other mouse strains and their macrophages are highly resistant to LT. The underlying mechanism of resistance remains unknown and is a subject of great interest in the anthrax field due to the potential usefulness of this information to development of therapeutics. The primary goal of this project is to investigate mechanisms of resistance to LT in macrophages. To address this goal, three specific aims are proposed: (1) Investigation of alterations in cellular trafficking and localization using immunofluorescence/confocal microscopy to examine localization of LF in sensitive versus resistant macrophages, (2) Investigation of the role of potential LF intracellular substrates in resistant macrophages, and (3) Analysis of differential microarray analyses to evaluate gene expression in LT-sensitive versus resistant macrophages with a focus on evaluating cytokine expression, MAPK kinase effector molecule expression, and expression of Kif1C, a recently described murine gene product associated with resistance to LT. Successful completion of these studies will yield a fundamental understanding of mechanisms of macrophage resistance to LT.

Grant: 1R21AI056134-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: CHAN, JOANNE PHD
Title: Attacking anthrax action by blocking receptor signaling
Institution: DANA-FARBER CANCER INSTITUTE BOSTON, MA
Project Period: 2003/09/30-2005/08/31

DESCRIPTION (provided by applicant): Application for R21, NOT-AI-02-023, Biodefense and Emerging Infectious Diseases Research Anthrax toxin, isolated from the bacterium, *Bacillus anthracis*, enters human cells and disrupts cellular function. The toxin consists of a receptor binding component, protective antigen (PA) which can associate with the enzymatic components, edema factor (EF) and/or lethal factor (LF) to form the anthrax toxin. The toxin binds a cell surface receptor which mediates the internalization of the toxin complex. Recently, work from the laboratories of John Young and John Collier identified the receptor for the anthrax toxin, named the anthrax toxin receptor (ATR; Bradley et al., 2001). Since the ability of the anthrax toxin to cause harm is dependent upon receptor binding, targeting the anthrax receptor might provide additional therapies that could be useful even after suspected exposure to anthrax spores. In many cases, signaling downstream of cell surface receptors involves the activation of protein and lipid kinases. Successful use of the small molecule kinase inhibitor Gleevec in chronic myelogenous leukemia has shown that targeting kinases might provide an efficient means to treat various diseases. This goal of this project is to study the ATR signaling pathway in order to identify key effector molecules as targets for inhibition. The role of ATR in mediating toxin internalization will be dissected using biochemical and immunofluorescence methods in mammalian cells. The zebrafish will be used as an animal model to study ATR function in vivo and to assess its potential as an animal model for testing drugs aimed at blocking toxin action. The applicant has 3 specific aims which will be undertaken in collaboration with the Young and Collier labs. Aim 1. To investigate the role of the cytoplasmic domain of the anthrax receptor for potential therapeutic intervention. Aim 2. To determine the physiological role of the anthrax receptor by functional interference during normal embryonic development in the zebrafish. Aim 3. To examine anthrax toxicity in the zebrafish for use as an additional inexpensive model system for drug or vaccine testing.

Grant: 1R21AI056161-01
Program Director: BEANAN, MAUREEN J.
Principal Investigator: FLAJNIK, MARTIN F PHD
Title: Highly Stable, Anthrax-specific Shark Antibody Fragment
Institution: UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD
SCHOOL
Project Period: 2003/07/15-2005/06/30

DESCRIPTION (provided by applicant): There is an urgent demand for the development of innovative diagnostic and therapeutic products for widespread use in the bio-defense field, especially against Category A pathogens such as *Bacillus anthracis*. To this end it is desirable that the developed products show high specificity for their target and high stability to denaturation. Recent work identified a novel antibody isotype, called IgNAR (novel antigen receptor) in the serum of nurse sharks. This molecule is unusual as the variable (V) regions, responsible for binding antigen, did not form a dimer as in conventional antibodies but are able to bind antigen as individual soluble domains. Due to its high level of targeted somatic hypermutation IgNAR appears to be the molecule responsible for the true adaptive immune system in cartilaginous fish. The aim of this project is to isolate specific and highly stable single domain antibody fragments from nurse sharks immunized with the recombinant form of protective antigen (rPA) from *B. anthracis*. Due to the unique nature of these molecules, if this initial antigen proves successful, we envisage this technique will provide a platform technology which could easily be utilized to supply similar fragments for use as diagnostic or therapeutic tools for limitless other targets of interest for bio-defense.

Grant: 1R21AI056207-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: MCCORMICK, JOSEPH B MD
Title: EMERGING MDR TUBERCULOSIS ACROSS THE US/MEXICO BORDER
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX
HOUSTON
Project Period: 2003/09/15-2005/08/31

DESCRIPTION: (provided by applicant) Our application will create dynamic databases to analyze and generate preliminary data on the precise patterns of transmission of tuberculosis (TB), especially multidrug resistant tuberculosis (MDR-TB), across South Texas and North Eastern Mexico. This will be achieved through a binational consortium that will share social, demographic, and geographic data on tuberculosis cases from each of their respective areas. The aims of the consortium are 1) aggregate existing sociodemographic, drug resistance and molecular fingerprinting databases currently held separately by consortium members 2) merge, geocode and analyze the aggregated database 3) establish common protocols and procedures for testing of drug resistance, extraction of *M. tuberculosis* DNA, and molecular characterization of isolates 4) evaluate social network analysis as a method for improving the tracking of MDR-TB transmission. These analyses link individual cases over 3-5 years with molecular fingerprints, drug susceptibility patterns, sociodemographic characteristics and geographic location, allowing regional understanding of the distribution and dynamics of DR and MDR-TB transmission across the border. These data will be used to improve control techniques and become the basis of hypotheses that will be tested in future, more elaborate research applications. These applications will include epidemiologic and translational research studies using common protocols for binational projects in emerging diseases, specifically MDR-TB. The geographic area of the consortium encompasses 5 counties of the Lower Rio Grande Valley (LRGV) from the Gulf of Mexico to Laredo, and the Mexican border states of Tamaulipas and Nuevo Leon. The consortium will be led by the Dean and faculty of the University of Texas School of Public Health Regional Campus at Brownsville, less than a mile from the border and contributing expertise in molecular microbiology, epidemiology, biostatistics, social network studies and spatial-temporal analyses. Leading tuberculosis researchers in Houston, San Antonio and Monterrey will play key roles in strengthening the capacity to conduct specialized public health, epidemiologic, and molecular based research on MDR-TB in the study area. Border consortium participants are from all the public health investigatorites responsible for TB elimination in both countries. An important defense of MDR introduction by bioterrorism is understanding how transmission of these organisms may differ from non resistant TB (if at all) and detailed knowledge of local strains of MDR that will allow quick distinction between known circulating strains of MDR TB and newly introduced strains. This application will be instrumental in addressing all of these issues.

Grant: 1R21AI056227-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: RAMAKRISHNAN, GIRIJA PHD
Title: Iron Transport Mechanisms of Francisella tularensis
Institution: UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA
CHARLOTTESVILLE
Project Period: 2003/09/15-2005/08/31

DESCRIPTION (provided by applicant): Francisella tularensis is a gram-negative bacterium and the causative agent of tularemia, a potentially deadly disease. It is a threat as an agent of bioterrorism because of the highly infectious nature of the airborne organism, with as few as 10 organisms being sufficient to cause disease. In the mammalian host, the bacterium is an intracellular pathogen, replicating within membrane-bound phagosomes in macrophages and other cells. Iron is a scarce but essential nutrient for the invading pathogen, and iron acquisition systems are proving to be critical virulence determinants. The availability of iron was shown to be important for proliferation of F. tularensis within macrophages by Fortier et al. (Inf. Immun.63:1478, 1995). Iron acquisition mechanisms in this organism are however uncharacterized. The goal of this proposal is identification of systems for iron acquisition in F. tularensis with the hypothesis that iron acquisition is a key element in pathogenesis in the mammalian host. A variety of techniques including bioinformatics (based on the genome sequencing project currently underway), proteomic and genetic approaches will be taken to achieve this goal. Specific aim 1 of this project is the characterization of iron utilization in the organism. By analyzing growth of the bacteria under conditions of iron-limitation, characterizing uptake of ferrous versus ferric iron, and determining the various host sources of iron (transferrin, heme etc.) that can be utilized, the physiologically significant sources of iron for the invading pathogen may be identified. These studies will help define assay methods for studying specific transport mechanisms. Specific aim 2 is the identification of iron acquisition systems and characterizing their role in virulence. Identification of potential transport systems will involve bioinformatics-based and proteomic approaches followed by targeted mutagenesis. Mutants will be tested for their ability to proliferate in a macrophage infection assay as a first level determination of their role in virulence. The long-term goals with these studies are to explore potential new mechanisms for iron acquisition, to be able to harness them in therapeutic applications, and to test them as targets for a defined vaccine strain development.

Grant: 1R21AI056229-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: SCANDURRO, ALINE B PHD
Title: Discovery of New Cellular Targets of Anthrax Toxin
Institution: TULANE UNIVERSITY OF LOUISIANA NEW ORLEANS, LA
Project Period: 2003/09/30-2005/08/31

DESCRIPTION (provided by applicant): The post- September 11 release of anthrax spores resulted in five civilian deaths, eighteen infections, and required that more than 30,000 individuals undergo prophylactic antibiotic therapy. This event also highlighted the need for a more thorough understanding of the pathogenesis of anthrax, and improved vaccines that would be appropriate for pre- or post-exposure immunization of civilian and military populations. The interaction of macrophages with one of the principal toxins produced by *Bacillus anthracis*, Lethal Toxin, has been a primary focus of laboratories studying the pathogenesis of this organism. However, we now know that dendritic cells are important sentinel cells distributed throughout the body that play a major role in detecting invading pathogens and in the initiation of the host immune response to foreign antigens. Our preliminary studies have shown that anthrax toxin affects cell signaling pathways and cytokine expression in toxin-treated dendritic cells. We will test the hypothesis that anthrax toxin targets several unknown but key molecular pathways within the dendritic cell that disarm its critical function in host-pathogen defense. The first specific aim is to determine the molecular changes within dendritic cells treated with anthrax toxin. We will use both gene microarray analysis and traditional cell signaling kinase assays to learn about both genomic and proteomic changes elicited by toxin treatment of these cells. The second aim is to determine the changes in cytokine expression profiles by dendritic cells treated with anthrax toxin. This will be measured by cytometric bead arrays, ELISA and RNase protection assays and is used here to reveal how the anthrax toxin-treated dendritic cell might affect other host cells and in particular those involved in host immune responses. This combined approach will significantly advance our understanding of *B. anthracis* pathogenesis in two ways: it will elucidate the dendritic cell's role in *B. anthracis* pathogenesis and it will lead to the identification of new cellular targets of anthrax toxin. Moreover, because of the critical role that dendritic cells play in initiating the host immune response, the proposed studies may lead to the development of improved vaccines against anthrax. This body of information will also serve as the foundation for new research areas to explore in future proposals.

Grant: 1R21AI056275-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: GOLDFINE, HOWARD PHD BIOLOGY
NEC:BIOCHEMISTRY
Title: Escape of Bacillus anthracis from the phagosome
Institution: UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA
Project Period: 2003/09/30-2005/08/31

DESCRIPTION (provided by applicant): In the pulmonary form of anthrax caused by the pathogen *Bacillus anthracis* (Ba), spores in the lung are ingested by macrophages. Once phagocytosed, the spores are transported to regional lymph nodes and germinate inside macrophages. In order for the disease to progress, Ba must resist killing by the macrophage and further disseminate into the blood for vegetative growth. The exact means by which Ba survives and escapes the macrophage are unknown. Recent work has shown that newly vegetative bacilli escape from the phagocytic vesicles of macrophages and replicate in the cytosol, a process that was first described and best studied in another human pathogen *Listeria monocytogenes* (Lm). Lm escapes from the primary phagocytic vacuole of a macrophage using listeriolysin O (LLO) and a phosphatidylinositol-specific phospholipase C (PI-PLC). Genes orthologous to LLO and PI-PLC have recently been discovered in Ba by the genome sequencing project. In order to investigate the functions of the Ba orthologs of LLO and PI-PLC, Lm will be used as a heterologous host to express these proteins and to analyze their role in mediating bacterial escape from phagocytic vesicles and release from the host cell. For safety reasons a strain of Lm that has been developed as a potential vaccine vector and is unable to grow in cells without a D-alanine supplement will be used. The first aim is to determine if the LLO ortholog permits escape of Lm from the macrophage phagosome. Its potential for signaling through a recently discovered protein kinase C signaling pathway, thought to be needed for escape from the phagosome, will also be explored. Since the Ba PI-PLC ortholog is 94% identical in amino acid sequence to the PI-PLC from *Bacillus cereus*, it almost certainly has that enzymatic activity, but there are potential structural differences between the *Bacillus* enzymes and the Lm enzyme that would affect their activity both outside and inside the host. The effects of these structural differences will be examined in the second aim, in which the role of Ba PI-PLC in escape from the phagosome and host cell signaling is explored. Lastly, studies on inhibitors of PI-PLC will test their efficacy in blocking the biological role of this enzyme and may lead to eventual high throughput screening for drugs to combat anthrax infections. The results of this study will provide evidence on the potential role of the LLO and PI-PLC orthologs of Ba in mediating its survival, growth and escape from the macrophage, essential elements in its ability to cause a devastating disease with high mortality in humans.

Grant: 1R21AI056278-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: MANN, BARBARA J
Title: Adhesins and Invasins of *Francisella Tularensis*
Institution: UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA
CHARLOTTESVILLE
Project Period: 2003/09/15-2005/08/31

DESCRIPTION (provided by applicant): *Francisella tularensis* causes a potentially life-threatening disease called tularemia. This pathogen can be aerosolized, survive in the environment and has an infectious dose of as few as 10 organisms. This has led the CDC and NIAID to classify this organism as a category A select agent and a potential agent of biological warfare. *F. tularensis* is a gram-negative coccobacillus that can infect a wide variety of vertebrate and invertebrate hosts. In mammalian hosts it primarily lives and replicates inside macrophages. The mechanisms by which the organism colonizes, invades and survives inside the host cell are mostly unknown. We have chosen to focus this proposal on microbial adherence since for many pathogens this is a key step in pathogenicity. Our overall hypothesis is that adhesions are critical virulence factors that are required for pathogenicity. A search of the genomic sequences of the virulent strain *F. tularensis* Schu4 has identified several loci with homology to genes involved in type IV pili structure and biogenesis. In other bacteria, which have a similar type of pilus, these pili have been shown to be critical virulence factors. This has led to our specific hypothesis: Type IV pili mediate attachment to host cell macrophages and help to facilitate host cell invasion. In this proposal we will focus on identifying and characterizing the gene(s) encoding pilin, the structural subunit of type IV pili, and investigating the role of this pilus in adherence, invasion and twitching motility. Specifically we will verify the presence of type IV pili by microscopy and immune sera. We will isolate pili from *F. tularensis* and sequence the pilin subunit by mass spectrometry analysis. We will purify pili for antibody production. To explore the diversity of the pilin subunit we will also clone and sequence the pilin encoding genes from the live vaccine strain (LVS) and additional clinical isolates of *F. tularensis*. We will develop an adherence and invasion assay using bacteria that express the gene encoding the green fluorescent protein. We will use pilin-specific antibody to try to block adherence and/or invasion. We will construct a pilin minus strain of *F. tularensis* and test its ability to adhere to and invade host cells and exhibit twitching motility. Understanding the basic mechanisms of pathogenicity will lead to a greater understanding of why this organism is such an effective pathogen, and the identification of new targets for therapies.

Grant: 1R21AI057188-01
Program Director: GIOVANNI, MARIA Y.
Principal Investigator: RELMAN, DAVID A
Title: Analytical Tools for Comparative Microbial Genomics
Institution: STANFORD UNIVERSITY STANFORD, CA
Project Period: 2003/08/15-2007/07/31

DESCRIPTION (provided by applicant): Genome microevolution in natural populations of pathogenic bacteria is accompanied by acquisition of virulence determinants and antibiotic resistance mechanisms, both of which constitute an ongoing threat to the public health. This diversity among genomes is also a powerful source of information for typing, tracking, and inferring the evolutionary history of bacterial pathogens. In particular, genomic comparisons of pathogenic and non-pathogenic strains of the same species can reveal the molecular basis of virulence. DNA microarray-based comparative genome hybridization (CGH) is a powerful, high-resolution tool for discerning molecular differences between related strains of sequenced bacterial species. Although the method has become increasingly popular, appropriate analytical methods for the interpretation of these data are lacking. Thus, the long-term goal of this proposal is to develop a set of computational tools to automate the analysis of CGH data and facilitate its interpretation in the context of the genome annotation. The first proposed program will perform two key tasks. Statistical classification of conserved and divergent sequences based on microarray hybridization intensity ratios will be accomplished using a mixture model. Inference of phylogenetic relationships between strains based on CGH data will proceed by maximum likelihood and generalized parsimony methods. The second program will be a visualization tool that integrates graphical representations of microarray data and genome annotation, and provides rapid search and retrieval of annotation data. Development of this tool will utilize the Java programming language and employ a wealth of available open source code. The third tool will be a database for storage, search, and retrieval of genome annotation and CGH data from multiple species. This database will be based upon the Genomics Unified Schema (GUS), an open source set of definitions and tools for combined storage of genome annotation and gene expression microarray data. Modifications to GUS will permit storage and linkage of CGH data. These three tools will be developed in a phased approach, capitalizing on the combined R21/R33 application mechanism. All tools will be freely distributed to the scientific community as they become available. In addition, the classification and phylogeny program will be offered as an on-line tool, and a *Bordetella* genome and CGH database will be built, maintained, and served to the community on-line.

Grant: 2R37AI015940-24A1
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: VASIL, MICHAEL L PHD
MICROBIOLOGY:BACTERIOLOGY
Title: Iron and O₂ in the Regulation of *P. aeruginosa* Virulence
Institution: UNIVERSITY OF COLORADO DENVER/HSC DENVER, CO
AURORA
Project Period: 1979/04/01-2007/11/30

DESCRIPTION (provided by applicant): *Pseudomonas aeruginosa* is a malicious opportunistic pathogen both in terms of the severity and the outcome of infections it causes. A significant proportion of patients with cystic fibrosis (CF) is colonized at an early age (1-2yrs), and most ultimately succumbs to a chronic lung infection from *P. aeruginosa*. The myriad of virulence determinants, including colonization factors and toxins that *P. aeruginosa* produces contribute to its pathogenic potential. Unfortunately, the exact contribution of these factors, alone or in combination, to even the simplest kind of *P. aeruginosa* infection has not yet been elucidated. Expression of all the identified major virulence determinants in this organism is regulated by a variety of environmental conditions, which the organism encounters at some point in its journey through the host. Consequently variations in available environmental iron undoubtedly contribute to the pathogenesis of *P. aeruginosa* infections. Production of specific virulence factors of this organism are induced in response to limiting amounts of iron, a natural occurring environment in mammalian hosts. The dynamic control of intracellular iron concentrations is paramount to all biological systems. One aspect of this issue is that, especially in an aerobic environment, biologically useful iron (i.e., Fe²⁺) is extremely limiting or it is highly insoluble (i.e. Fe³⁺). Accordingly, biological entities have evolved efficient mechanisms to acquire this nutrient from the insoluble form, which is generally in plentiful quantities. On the other hand, further acquisition of iron above biologically useful concentrations can have dire consequences for a cell. Excess free iron will catalyze the generation of highly reactive oxygen and nitrogen intermediates that will damage all known biological macromolecules. This conflict, in a major way is dealt with in a diverse array of pathogenic and commensal prokaryotic microbes, by repressor proteins, which play the key role in controlling iron homeostasis at the level of transcription. The ferric uptake regulator (Fur) serves this function in many bacteria. In fact, in the opportunistic pathogen *P. aeruginosa* Fur (PA-Fur) is an essential protein that controls the expression of genes involved in the acquisition of environmental iron, including those that contribute to its virulence. This project will investigate the role of PA-Fur and PA-Fur regulated genes in the pathogenesis of *P. aeruginosa* infections for the ultimate goal of developing novel antimicrobial agents against this formidable opportunist.

Grant: 2R37AI016935-19
Program Director: HALL, ROBERT H.
Principal Investigator: PAYNE, SHELLEY M PHD MICROBIOLOGY, OTT
Title: Iron transport in Shigella and pathogenic E.coli
Institution: UNIVERSITY OF TEXAS AUSTIN AUSTIN, TX
Project Period: 1980/09/30-2007/11/30

DESCRIPTION (provided by applicant): Most bacterial pathogens have an absolute requirement for iron. The low availability of iron in most environments has led to the evolution of high affinity iron transport systems. Although a variety of iron transport systems have been identified in Shigella and pathogenic E. coli, the sources of iron used by the pathogens when growing within the host and the specific iron transport systems involved in growth and survival in vivo are not known. Shigella species, the causative agents of dysentery, are closely related to E. coli and share many of the same iron transport systems. However, differences have been noted among this group of pathogens and those differences may relate to differences in sources of iron at various sites within the host or in the environment. Because Shigella spp. have the ability to invade host cells and grow with the cytosol, there may be specific iron transport systems associated with iron acquisition in the intracellular environment. Our first specific aim is to complete the characterization of the Shigella heme transport systems. Many shigellae and pathogenic E. coli, including O157:H7, have specific receptors for heme. Although receptors and other proteins involved in transporting heme across the bacterial cell wall have been identified, other steps in heme acquisition and its use as an iron source are not understood. The second aim is to use genetic and genomic approaches to identify the additional iron uptake systems in these pathogens. We will then apply these data and use the mutants created in these studies to help understand the role of the iron transport systems in growth and survival in vivo and in the environment. Thus our third specific aim is to determine which systems are used under specific environmental conditions and during intracellular growth. Our fourth specific aim is to assess expression of iron transport and other genes during intracellular growth and during infection by using microarray technology.

Grant: 2R37AI033493-10
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: SEIFERT, H STEVEN
Title: Mechanisms of Gonococcal Pilin Antigenic/Phase Variation
Institution: NORTHWESTERN UNIVERSITY CHICAGO, IL
Project Period: 1994/05/01-2008/01/31

DESCRIPTION (provided by applicant): The Gram-negative bacterium, *Neisseria gonorrhoeae*, is the only causative agent of the sexually transmitted disease gonorrhea. One important facet of gonococcal pathogenesis is the process of pilin antigenic variation (Av), which allows for immune evasion by the bacterium and continual retransmission through the high risk/core group of infected people. In addition, the pilus functions in the initiating events of colonization and the functional properties of the pilus can be altered by pilin antigenic variation. Understanding the mechanisms used to effect and control pilin Av is essential to understanding this aspect of the pathogenesis of gonococcal disease. This proposal will continue our genetic studies into the molecular mechanisms used to enable pilin Av. There are two specific aims: Aim 1 will continue our identification and characterization of genes and protein products, revealed by a genetic screen, that are involved in mediating and regulating pilin antigenic variation. Aim 2 will explore the mechanisms of pilin antigenic variation by defining the role of conserved DNA sequences in pilin antigenic variation, by testing novel models that explain pilin antigenic variation, by defining how variability of pilin variation is maintained, and by re-examining a controversial hypothesis that DNA transformation has a role in mediating pilin Av. The results from these studies will help maintain pilin antigenic variation as one of the main prokaryotic paradigms for systems based on genomic translocations, and further our understanding of how DNA recombination can be central to an important mechanism of microbial pathogenesis.

Grant: 1U01AI054374-01
Program Director: GIOVANNI, MARIA Y.
Principal Investigator: HENRICKSON, KELLY J MD
Title: Multiplex PCR Detection of CDC 'A' Bioterrorism Agents
Institution: MEDICAL COLLEGE OF WISCONSIN MILWAUKEE, WI
Project Period: 2003/04/01-2006/03/31

DESCRIPTION (provided by applicant): Anthrax and other agents of biological warfare have recently received intense publicity. These weapons are an increasingly fearsome danger to our civilization. Agents identified by the CDC (category "A") to pose the greatest threat include Variola major (smallpox), Bacillus anthracis (anthrax), Yersinia pestis (plague), Clostridium botulinum toxin (botulism), Francisella tularensis (tularemia), and a group of RNA viruses that cause hemorrhagic fevers (VHFs, e.g., Ebola). Accurate and efficient techniques to identify and diagnose these agents are severely limited. This lack of good diagnostic tests hampers the majority of goals set forth by the NIAID and CDC to prepare the U.S. to counter future bioterrorism attacks. Available older techniques have proven unreliable. Modern molecular tests like individual PCR assays have been developed for some agents. These offer increased speed and sensitivity but because there are so many bioterrorism agents it is prohibitive to run dozens of "singleplex" arrays on each specimen. Similarly, recently reported microchip (MAGI Chip) arrays and other microarrays suffer from either needing PCR amplification first, or from the high cost to make the arrays, and the need for sophisticated equipment. A single assay (or two) that could detect a large number of bioterrorism agents rapidly, sensitively, specifically, and cheaply would greatly enhance antiterrorism planning and biodefense. Our laboratory has pioneered a method of multiplex PCR that can accomplish this goal. This proprietary method (two U.S. patents) has been used commercially in the Hexaplex(r) Assay, which can detect seven common respiratory viruses in a single test. The Specific Aims of this project are: 1) To determine if a multiplex PCR-enzyme hybridization assay (EHA) can be made using our unique technology that will identify all of the CDC Category "A" Bioterrorism agents that are DNA based; 2) RNA based; and finally 3) a single combined multiplex (RNA/DNA) PCR assay with an analytical sensitivity equal to "singleplex" real time assays as developed by the CDC. Specific Aim 4: To determine if this multiplex assay is equivalent to these "singleplex" assays in a clinical trial.

Grant: 1U01AI054641-01
Program Director: ZOU, LANLING
Principal Investigator: MILLER, BARBARA A PHD
Title: Non-Invasive Plant Virus Particle-Based Anthrax Vaccines
Institution: DOW CHEMICAL COMPANY MIDLAND, MI
Project Period: 2003/05/15-2007/04/30

DESCRIPTION (provided by applicant): Recent anthrax threats to Homeland Defense demand new vaccine technologies. Problems with the current licensed anthrax vaccine include variable immune response, adverse reactions and high production costs. In addition, significant uncertainties exist regarding the efficacy for inhalation anthrax and the schedule and route of administration are suboptimal. The proposed work targets the development of a low-cost, safe and effective, non-invasive anthrax vaccine based on the plant transient gene delivery vector (TGDV) platform. This proposal tests the hypothesis that TGDVs can be used as particulate carriers of selected antigenic peptides derived from anthrax Protective Antigen (PA). The Dow team proposes that TGDV-based vaccines can be produced in plant tissues in large volumes and shorter time at significantly lower cost and minimal capital investment. The team further proposes that TGDVs can be formulated for non-invasive administration and due to their particulate nature provide enhanced protective immunity while minimizing side effects without the need for an adjuvant. Three different TGDVs that accommodate varying sizes of target peptides and assemble into different particulate shapes will be used to express four different regions derived from the PA. The constructs will be propagated in plants and used for immunology studies. The specific aims of this proposal are (1) engineering and cloning of antigenic determinants from the anthrax PA into expression vectors; (2) optimizing, producing and purifying recombinant TGDV's fused with target peptides, (3) testing immunogenicity in vitro using dendritic cell assays; (4) testing immunogenicity of selected constructs in vivo using nonhuman primates; and (5) conducting challenge studies in nonhuman primates. The new strategic partnership among The Dow Chemical Company (Dow), the Fraunhofer U.S.A. Center for Molecular Biotechnology, The University of Maryland, and Ohio State University (Consultant) leverages expertise in manufacturing excellence, virology, immunology, and anthrax pathogenicity respectively. The partnership, in collaboration with NIH scientists offers advancement of this novel technology platform and ultimate commercialization of new vaccines of public health importance.

Grant: 1U01AI054774-01
Program Director: ZOU, LANLING
Principal Investigator: LIN, AUGUSTINE Y PHD
Title: Development of a Novel Retrogen Vaccine for Anthrax
Institution: MITHRAGEN, INC. HOUSTON, TX
Project Period: 2003/04/04-2004/03/31

DESCRIPTION (provided by applicant): Anthrax is a fatal septicemic disease caused by ingestion or inhalation of *Bacillus anthracis* spores. Once infection is established, mortality rates may approach 90%, making *B. anthracis* a weapon of choice among bioterrorists. The only anthrax vaccine licensed for use in the US is plagued with problems related to low immunogenicity and a relatively high level of adverse side effects. This Phase I proposal seeks to outline the methods by which a safe, efficacious, and cost-effective DNA vaccine may be developed for the widespread prevention of anthrax disease among the general public. Various *B. anthracis* toxin subunits (PA, EF, and LF) and spore surface antigens (EA1, Sap, CapA, CapB, CapC, and Dep) will be tested in conjunction with Retrovax, a proprietary vaccine technology which exponentially enhances dendritic cell antigen presentation, inducing a sustained CD4+ T cell response in addition to the CD8+ T cell and antibody responses typical of DNA vaccination. This robust CD4+ response, unique among current cell-free delivery systems, should coordinate the CD8+ T cell mediated clearing of infected macrophages presenting toxin subunit peptides on their MHC Class I molecules in the early stages of anthrax infection. Humoral responses to spore surface antigens offer the ability to clear dormant anthrax spores prior to germination. Humoral responses to toxin subunits should neutralize toxin activity from any infected macrophages, which escape early immune surveillance.

Grant: 1U01AI054778-01
Program Director: AULTMAN, KATHRYN S.
Principal Investigator: FEDERICI, BRIAN A PHD
Title: Highly Improved Bacterial Larvicides for Vector Control
Institution: UNIVERSITY OF CALIFORNIA RIVERSIDE RIVERSIDE, CA
Project Period: 2003/04/15-2008/03/31

DESCRIPTION (provided by applicant): The objective of the proposed research is to develop a series of highly improved recombinant bacterial larvicides for controlling the mosquito vectors of major human diseases including malaria, filariasis, dengue, and the viral encephalitides. These bacteria will be significantly more cost-effective than *Bacillus thuringiensis* subsp. *israelensis* (Bti) and *Bacillus sphaericus* (Bs), the active ingredients of current commercial bacterial larvicides used in vector control. In addition, these recombinants will be much less prone to induce mosquito resistance, which has already developed to *B. sphaericus* in *Culex* populations in Brazil, China, and India. Preliminary studies show that the production of high levels of the Bs2362 binary protein in Bti results in a recombinant strain tenfold more effective than the parental strains from which it was derived. This demonstrates that highly improved bacteria can be engineered for use in vector control. Despite these encouraging results, the development of recombinant bacterial larvicides is plagued by several major hurdles including low returns on investment, limited market size, and additional regulatory burdens imposed on recombinant organisms that result in long product development times. The present project is a collaboration between the University of California, Riverside and Valent BioSciences of Libertyville, Illinois, that will combine the expertise of both parties to accelerate the development highly improved bacterial larvicides for vector control. Through focused engineering research, a series of much more effective strains of Bti and Bs based on Cry, Cyt, and Bs mosquitocidal proteins will be developed and commercialized that also meet U.S. regulatory requirements. Improved strains using Bs as a host cell will be developed primarily for control of *Culex* species (vectors of filariasis and West Nile Virus) breeding in semi-polluted and highly polluted waters, whereas Bti will be used as the host cell for control of *Aedes* species (vectors of dengue) and certain anopheline vectors of malaria. This collaboration has the common goal of having new products based on these recombinant bacteria on the market within five years, sooner if possible.

Grant: 1U01AI054782-01
Program Director: SCHMITT, CLARE K.
Principal Investigator: OBRIG, TOM PHD
Title: NEW THERAPEUTICS FOR PATHOGENIC E-COLI DISEASES
Institution: UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA
CHARLOTTESVILLE
Project Period: 2003/03/01-2008/02/29

DESCRIPTION (provided by applicant): Pathogenic *E. coli* are a major cause of human diseases. *Escherichia coli* 0157:H7 is an "emerging infectious disease" responsible for outbreaks of food-borne illness and is the leading cause of hemolytic uremic syndrome (HUS), acute renal failure in young children. There is growing evidence that inflammatory mediators are induced in the host by *E. coli* 0157:H7 LPS and Shiga toxins and that both are required to cause HUS. In addition, Shiga toxins (Stx1, Stx2) produced by *E. coli* 0157:H7 are required for the vascular complications in HUS. At present, no effective treatments are available for *E. coli* 0157:H7. Thus, our long-term goal is to develop therapeutic modalities based on mechanisms by which LPS, Stx1, and Stx2 cause disease. The short-term goal is to define host inflammatory responses to *E. coli* LPS and Shiga toxins. In the present study, gene microarrays are employed to delineate the inflammatory response to LPS and Stx2 in mouse models of kidney and lung disease. In addition, the effectiveness of anti-inflammatory adenosine A2A receptor agonists will be tested in the murine model of LPS plus Stx1/Stx2-induced inflammation. These latter studies are based on two recent findings: 1) that a pronounced inflammatory phase is associated with LPS/Stx2 response in the kidneys of mice, and 2) that A2A receptor agonists exhibit a protective role in LPS-dependent lung inflammation and prevent LPS-induced mortality. Application of the anti-inflammatory A2A receptor agonists to *E. coli* 0157:H7-associated disease will be further defined using A2A receptor deficient mice and adoptive bone marrow transfer to determine the role of circulating inflammatory cells in this disease.

Grant: 1U01AI054785-01
Program Director: ZOU, LANLING
Principal Investigator: DAVID, SUNIL A MD
Title: Hydrophobic Polyamine Amides as Anti-Endotoxin Agents
Institution: UNIVERSITY OF KANSAS LAWRENCE LAWRENCE, KS
Project Period: 2003/05/20-2008/04/30

DESCRIPTION (provided by applicant): Lipopolysaccharides (LPS) or endotoxins are outer membrane constituents of gram-negative bacteria that play a key role in the pathogenesis of septic shock, a leading cause of mortality worldwide for which there is as yet no effective therapy. The pathogenesis of certain Category A (*Francisella tularensis*) and Category B (*Brucella* spp.) bioterrorism agents also involves deleterious host responses to LPS. One possible approach to developing novel therapeutic strategies to treat sepsis is to sequester circulating LPS, a strategy that has been historically addressed using monoclonal antibodies directed against the structurally conserved lipid regions of LPS. However, a series of clinical trials using monoclonal antibodies have been unsuccessful owing to the lack of accessible recognition sites on the lipid. Our previous work on identifying structural requisites necessary for binding and neutralization of LPS in a variety of proteins, peptides and small molecules led to the identification of a novel class of structurally simple, nontoxic molecules, the lipopolyamines, which bind and neutralize LPS in vitro, and afford protection against LPS challenge in two murine models of gram-negative sepsis. In collaboration with MediQuest Therapeutics, Inc., we propose to synthesize libraries of novel compounds rationally designed to maximize binding affinity and neutralization potency, and to exhibit desirable pharmacokinetic and toxicological profiles, based on optimal structural templates that we have already established with the lipopolyamines. Employing a hierarchical screening strategy, the interactions of these molecules with LPS will be comprehensively evaluated. Test compounds will be screened for the ability to inhibit LPS-induced cellular activation and production of key proinflammatory mediators of septic shock. Highly active molecules will be further tested in two murine models of gram-negative sepsis. The toxicity of the compounds will be systematically determined in a panel of in vitro assays. These studies will serve to generate data for anticipated IND submissions.

Grant: 1U01AI054815-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: KIMMEL, BRUCE E BA
Title: Characterization of Proteomes of Category A Pathogens
Institution: DIVERSA CORPORATION SAN DIEGO, CA
Project Period: 2003/08/01-2006/01/31

DESCRIPTION (provided by applicant): The objective of the research described in this proposal is to identify new/better protein targets for vaccines, diagnostic antibodies and therapeutic antibodies against *B. anthracis* and *Y. pestis*. In addition, Diversa will apply its unique protein engineering capabilities toward isolating and optimizing diagnostic and therapeutic antibodies for better biodefense. Specific Aim 1: Characterize the proteomes of *B. anthracis* and *Y. pestis* using a proteomics approach. Specific Aim 2: Genetic analysis of proteins that are correlated with pathogen virulence. Specific Aim 3: Build a fully synthetic human antibody library & optimize a FACS-based screening approach. Using a state-of-the-art proteomics platform based upon micro 3D liquid chromatography, Diversa will identify novel proteins and measure protein expression level differences from samples of *B. anthracis* and *Y. pestis* that have been grown under various conditions, as well as coordinate (in collaboration with Dr. Arthur Friedlander (USAMRIID) and Dr. Matt Niles (U. of N. Dakota) a genetic analysis of surface and secreted proteins that are characterized in the proteomics analysis, in order to further the understanding of pathogenesis to allow better vaccines or treatments to be developed. This proposal also outlines the development of an in vitro immune system that will be independent of animal immunizations and phage display. First, Diversa will construct a synthetic human antibody library from the human genome using a proprietary process called GeneReassembly. Second, Diversa will advance high throughput screening technologies for isolating antibody leads from the library. Third, Diversa will apply its substantial expertise in protein engineering to improve antibody properties. Using its patented Gene Site Saturation Mutagenesis, Diversa will improve key properties of antibody candidates, such as binding affinity, thermal stability, solubility, and recruitment of effector functions. The development of this in vitro immune system has strong potential to create a process that generates a larger number of candidate antibodies per target and delivers candidate therapeutic antibodies to the clinic faster than current approaches.

Grant: 1U01AI054816-01
Program Director: PERDUE, SAMUEL S.
Principal Investigator: WALSH, SCOTT PHD BIOMEDICAL ENG
Title: PEGylated Lysostaphin for Staphylococcal Infections
Institution: BIOSYNEXUS, INC. GAITHERSBURG, MD
Project Period: 2003/07/15-2005/06/30

DESCRIPTION (provided by applicant): Lysostaphin is a 27 kDa enzyme that cuts the pentaglycine cross-link in the cell wall peptidoglycan of *Staphylococcus aureus*. We have shown that lysostaphin is extremely effective in treating *S. aureus* infections in animal models and that in the presence of low levels of beta-lactam antibiotics the development of resistance to lysostaphin is not seen in *S. aureus*. However, in animal models, infused lysostaphin is rapidly cleared from the circulation, and repeated administration induces the production of anti-lysostaphin antibodies that could reduce its therapeutic effectiveness. The aim of this project is to improve the circulating half-life of lysostaphin. We propose to develop poly(ethyleneglycol)-conjugated lysostaphin that retains its bactericidal and therapeutic activity and shows reduced clearance by glomerular filtration and other routes and reduced immunogenicity. Improving the half-life of lysostaphin in circulation should reduce both the dosing quantity and frequency required to maintain plasma concentrations above therapeutically effective levels. Maintaining prolonged high levels of lysostaphin may also result in more rapid clearance of bacterial infections. Lysostaphin promises to be an invaluable tool to healthcare professionals in combating the occurrence of nosocomial *S. aureus* infections (as a nasal cream) and in treating topical and systemic *S. aureus* infections, including *S. aureus* endocarditis.

Grant: 1U01AI054842-01
Program Director: LAUGHON, BARBARA E.
Principal Investigator: DICK, JAMES D PHD
Title: Drug Development for MDR-M tuberculosis
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 2003/03/15-2008/02/29

DESCRIPTION (provided by applicant): Tuberculosis is one of the oldest and most common infectious diseases of man. The disease is epidemic with approximately 1.8 billion people or one third of the world's population currently infected. The HIV epidemic and the emergence of multi-drug resistant *M. tuberculosis* (MDR-TB) has led to a critical need for the discovery and development of new and effective therapeutic agents for the treatment and control of tuberculosis. The b-sulfonylcarboxamides are a novel class of compounds which have potent in vitro activity against pathogenic mycobacteria with minimal inhibitory concentrations (MICs) ranging from 0.78 micrograms per ml to 12.5 micrograms per ml for *M. tuberculosis*, including MDR-TB. In addition, these compounds are highly specific for pathogenic mycobacteria, maintain activity in the presence of human serum, and are not cytotoxic for mammalian cells at 40 times their MIC for *M. tuberculosis*. Recent studies in our laboratories have demonstrated the antimycobacterial activity of the b-sulfonylcarboxamides to be the result of inhibition of a potentially unique pathway/target involved in central energy metabolism. The long-term objectives of this grant proposal are to determine the molecular target and mechanism of action of this novel class of compounds, with subsequent optimization of drug structure, synthesis, and preclinical drug development. This will be accomplished through: (1) characterization and identification of the target/pathway using available genomic and proteomic technologies, (2) confirmation and validation of candidate target(s) through microbiologic, biochemical, and genetic comparisons between resistant and susceptible mycobacteria, (3) determination of the three dimensional structure of the identified target, (4) design and synthesis of optimum small molecule inhibitors of the identified target/pathway (5) in vitro and in vivo toxicity and efficacy testing in a murine model of tuberculosis.

Grant: 1U01AI054889-01
Program Director: AULTMAN, KATHRYN S.
Principal Investigator: NOVAK, ROBERT J PHD
Title: Microbial Control of Immature Anopheles Mosquitoes
Institution: UNIVERSITY OF ILLINOIS URBANA- CHAMPAIGN, IL
CHAMPAIGN
Project Period: 2003/05/02-2008/04/30

DESCRIPTION (provided by applicant): The scientific goal of this research proposal is to demonstrate that larval management using environmentally safe and efficacious microbial insecticides in rice can provide an integral part of a malaria control program. This investigation is designed to develop larval management strategies and new microbial formulations that can provide additional entomological tools to reduce malaria in conjunction with adult control, personal protection, and clinical treatment. This proposal emphasizes attacking anopheline mosquitoes before they become airborne, when they are the most concentrated, immobile and accessible, or basic Integrated Mosquito Management (IMM). The greatest barrier to initiating a larval management program is that larval ecology of African malaria vectors is a neglected area of malaria research. Thus there has also been an associated lack of research in the development and deployment of environmentally safe microbial larvicides, such as *Bacillus thuriangiensis* var. *isrealensis* (Bti) and *Bacillus sphaericus* (Bsph). In order to develop, implement, and maintain a mosquito management program in rice irrigation schemes, we must determine site-specific IMM critical elements; the when, where, and how to reduce adult emergence by larval management. This study will provide both the efficacy data on the impact of microbial control and critical environmental data necessary for registration of new formulations both in the US and in international locations, thus circumventing prohibitive costs to industry. To accomplish this goal, the following specific aims have been developed. 1) determine the spatial and temporal distribution and abundance of *Anopheles* larval habitats and their vector productivity in irrigated rice-village complexes, 2) determine the key environmental, agricultural, and ecological factors that regulate vector productivity and non-target abundance and diversity in the larval habitats, 3) develop, evaluate and register new microbial-larvicide formulation(s) designed for effective residual control of anopheline larvae, and 4) implement and evaluate the impact of an area-wide mosquito management program on the entomological parameters associated with the transmission of malaria in rice.

Grant: 1U01AI054971-01

Program Director: BAKER, PHILLIP J.

Principal Investigator: KANTOR, FRED S

MD INTERNAL

MED:INTERNAL MEDICINE

UNSPEC

Title: Tick Vaccine Prevention of Lyme Borreliosis

Institution: YALE UNIVERSITY

NEW HAVEN, CT

Project Period: 2003/03/15-2007/02/28

DESCRIPTION (provided by applicant): Our strategy is to prevent transmission of Lyme borreliosis by interfering with the tick's ability to feed to repletion. The proposed project attempts to identify and characterize a family of anticoagulants used by Ixodes ticks needed to permit feeding on their mammalian hosts. Such anticoagulants are vital to the success of tick feeding and in the transmission on tick borne pathogens. We shall explore the family of Ixodes tick anticoagulants by biochemical separation from tick salivary gland and saliva, and by probing cDNA and genomic libraries with appropriate primer fragments. Previous attempts in our laboratories to isolate tick salivary proteins by probing cDNA libraries with antibodies from animals made tick immune resulted in 40 clones; 22 of which were represented by an anticoagulant that we have called SALP-14. In the present proposal we will probe libraries with primers derived from homologous portions of the clones previously identified in order to characterize what we now recognize as a family of anticoagulants vital to tick feeding and transmission of Lyme borreliosis. We will express and purify the recombinant anticoagulants and characterize their activities. To determine active sites, we will generate deletion mutants of these anticoagulants and identify the smallest region essential for effective catalytic activity, as well as antigenicity. The goal is to interfere with feeding by the immune response directed against the functional epitope(s) of the anticoagulant molecules. Feeding to repletion is essential for ticks to complete their life cycle and for the transmission of the pathogens they harbor.

Grant: 1U01AI055010-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: SYKES, KATHRYN F PHD
Title: Discovery of new anti-bacteremia vaccines for anthrax
Institution: MACROGENICS, INC. DALLAS, TX
Project Period: 2003/09/15-2006/02/28

DESCRIPTION (provided by applicant): Anthrax was developed as a biowarfare agent by the US government and a number of other countries. Despite treaties and resolutions to terminate these programs, anthrax remains a very real, world defense issue. Since an anthrax vaccine will be a critical component of any effective defense plan, our objective is to find one. Vaccine efforts for anthrax have focused almost entirely on improving the purification or formulation of PA or one of the other toxin antigens. We believe that vaccine components that target the spore or bacterium directly will be important in protecting both military and civilian populations against both natural and especially bio-engineered anthrax disease. Therefore, we propose to screen all the genes of *Bacillus anthracis* for their ability to protect against inhalation anthrax in mice. Our approach will be to build all anthrax ORFs as gene vaccines, and then design a matrix arraying system that allows us to screen the high complexity library down to a few vaccine candidates in one animal challenge experiment. The underlying functional genomic methods were developed with DARPA funding: expression library immunization and linear expression elements. These platform technologies make our approach technically feasible, and their utility has been validated in our labs. We are now poised for their application to anthrax. Specifically, our aims will be to generate the more than 4,000 genes of *B. anthracis* as more than 14,000 overlapping gene fragments that are capable of uniformly high-level expression. Using our unique technologies, vaccine candidates will be identified quickly. Immune assays will be used to direct the optimization of protection in rodents. Then, a set of our best candidates will be tested in rabbits against an inhalation anthrax challenge. At the completion of this project, we will deliver a set of broad-based vaccine candidates against anthrax that might be administered with or without toxin antigens. These will be ready for preclinical trials. We should also have in place a high-throughput antigen discovery system that could be applied to other biothreat and emerging pathogens.

Grant: 1U01AI056383-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: STEWART, DONALD I.H. PHD
Title: Antibodies to Burkholderia type III secretion system
Institution: CANGENE CORPORATION MISSISSAUGA, ON
Project Period: 2003/09/15-2006/02/28

DESCRIPTION (provided by applicant): *B. mallei* and *B. pseudomallei* ("B. (pseudo)mallei"), two closely related Gram-negative bacteria, are serious potential bioterrorist agents listed on CDC/MMWR's category B list. These organisms cause life-threatening infections (Glanders' disease and Melioidosis, respectively), where antibiotic treatment is sometimes insufficient. The virulence factors of the two species are not well understood, and it is difficult to distinguish these species from each other and also from the closely related avirulent species *B. thailandensis*. *B. (pseudo)mallei* is also a civilian problem causing septicemia in Southeast Asia. Another *Burkholderia* species, *B. cepacia*, is a more common civilian problem that increases mortality and morbidity of cystic fibrosis patients. To better treat and diagnose *B. (pseudo)mallei*, we will develop monoclonal antibodies (mAbs) against potential virulence factors: components of the type III secretion system (TTSS). The presence of TTSS has been correlated to virulence of *Burkholderia*. Also, since several other invasive Gram-negative bacteria utilize TTSS to enter host cells, it is plausible that TTSS are critical for *Burkholderia*'s virulence as well. TTSS components will be cloned and expressed in *E. coli* to generate sufficient quantities of purified antigen to generate mAbs. Fully human mAbs will be generated by phage display technology. Initial screening of the mAbs for reactivity will be conducted against a panel of *Burkholderia* isolates cultivated under different conditions, mAbs of interest will be further evaluated for protective capability in both in vitro and subsequently in vivo small animal models. We will also investigate potential differences of TTSS genes among isolates of *Burkholderia*. In summary the primary objectives of the project are: -Development of mAbs with utility in diagnosing and treating *B. (pseudo)mallei* and other *Burkholderia* species, including *B. cepacia*. -Improved understanding of the pathogenicity of *B. (pseudo)mallei* as a basis for potential vaccine development.

Grant: 1U01AI056385-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: STRONGIN, ALEX Y
Title: Develop Effective Inhibitors of Anthrax Lethal Factor
Institution: BURNHAM INSTITUTE LA JOLLA, CA
Project Period: 2003/08/01-2007/01/31

DESCRIPTION (provided by applicant): The purpose of this project is to develop, on an expedited schedule, those inhibitors of anthrax lethal factor (LF) which are both effective and safe for use by humans. The US government has forcibly stated that an effective treatment for anthrax is a key national priority. In response, the PI, in close coordination with the key collaborators, has developed a logical, closely interrelated program, which will lead to mature, drug-like compounds. This modular program employs the most current and advanced structural, enzymological, NMR and in silico methodologies. The development will be conducted through essential, seamlessly interacting modules with well-defined interfaces between complementary functions. The integral modules are: sieving the known, commercially available chemical structures by virtual ligand screening (flexible LF protein-ligand docking) to identify the limited number of potential inhibitors, testing the potency of the selected structures in enzymological and NMR studies, selecting via the in vitro and cell tests the inhibitors with minimal cytotoxicity and effect on human matrix metalloproteinases (MMPs), performing co-crystallization and structural analyses at the angstrom level of the inhibitor-LF complexes and the subsequent refinement of the chemical structures to accomplish the best fit. Following two or three iterations involving each of the above modules, the team will re-evaluate the lead compound(s) and perform the required benchmarks prior to moving the drug candidate(s) into clinical trials. Our five specific aims are: (I) establish a comprehensive panel of purified human MMPs; (II) examine the substrate preferences of LF and identify the structure of its potential peptide substrates and protein targets; (III) design the most potent and selective inhibitors capable of efficiently blocking the cleavage function of LF; (IV) test the potency of the derivative library of LF inhibitors selected in Aim III; (V) examine cytotoxicity and inhibitory potency of optimized, fine-tuned compounds, and to identify the drug-like lead inhibitor(s) of LF for continued drug refinement and clinical trials. We are confident that the resulting LF antagonist(s) will efficiently and safely provide the therapy that is so urgently required.

Grant: 1U01AI056431-01
Program Director: ZOU, LANLING
Principal Investigator: IVERSON, BRENT L
Title: Anthrax Antidote in Animals
Institution: UNIVERSITY OF TEXAS AUSTIN AUSTIN, TX
Project Period: 2003/08/05-2006/01/31

DESCRIPTION (provided by applicant): Anthrax remains a significant homeland security and military threat. The proposed work will deliver an optimized, anti-toxin antibody based therapeutic formulation that is tested through the non-human primate level. It will thus be ready for the final approval process and deployment. The whole IgG1 human form of our engineered 1H antibody will be produced and its therapeutic ability in a guinea pig spore challenge study will be investigated. Previously, we have shown that this ultra high affinity, anti-protective antigen (PA) antibody was extremely effective in an in vivo toxin neutralization assay using rats. The 1H work is being accelerated because we will need this data as a benchmark against which the other antibody formulations will be judged. In addition, given the current world tensions, it is prudent to have this promising therapeutic as far along as possible, as soon as possible. Note that if our results from the guinea pig spore challenge studies are as promising as we expect (and/or world events dictate), we will be in position to commence non-human primate trials of 1H within the first year. In addition to development of 1H, panels of ultra-high affinity antibodies will be produced to all three toxins in the first two years of the project. 1H is neutralizing because it disrupts the macrophage receptor-binding site of PA. Added therapeutic benefit may be realized by using additional antibodies that neutralize PA through disruption of binding to the lethal factor (LF) and edema factor (EF) toxins, as well as antibodies that prevent PA heptamerization. Further, the most effective strategy, and the one most likely to circumvent attempts to engineer resistance into new anthrax strains, may be to use a panel of ultra high affinity antibodies that have the redundant capacity to neutralize all aspects of the tripartite anthrax toxin activity. The most promising formulation of these engineered antibody panels will be determined by testing with anthrax spore challenge studies in guinea pigs, and ultimately, rhesus macaque monkeys, in collaboration with our subcontractor team led by Dr. Jean Patterson at the Southwest Foundation for Biomedical Research in San Antonio.

Grant: 1U01AI056443-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: RAO, VENIGALLA B PHD
Title: A Multicomponent Anthrax Vaccine using Phage T4 Display
Institution: CATHOLIC UNIVERSITY OF AMERICA WASHINGTON, DC
Project Period: 2003/08/15-2008/01/31

DESCRIPTION (provided by applicant): The goal of this application is to develop a multicomponent anthrax vaccine that can be easily administered, induces long-lasting high antibody titers, and provides protection against *Bacillus anthracis* infection. Three novel platform technologies, needle-free skin patch transcutaneous immunization (TCI), phage T4 multicomponent display, and liposome and emulsion adjuvant formulations, will be brought to bear on developing an efficacious anthrax vaccine. The research will be carried out by two complementary laboratories, one highly skilled in the genetics and manufacture of T4 bacteriophage particles and expression of proteins on the surface of T4, and the other with broad experience with TCI, immunogenic liposomes, emulsions, vaccine formulations, and vaccine clinical trials, in close collaboration with a third laboratory that is a leader in anthrax toxin biology and production. Mutant forms of all three anthrax toxin components, protective antigen (PA), lethal factor (LF) and edema factor (EF), will be expressed either as individual proteins or as N-terminal fusions of the highly antigenic outer capsid protein (Hoc) of bacteriophage T4. The toxin-Hoc fusion proteins with an N-terminal hexa-histidine tag will be purified in large quantities and loaded onto the phage T4 icosahedral surface using an in vitro assembly system. The TCI and intramuscular (IM) routes of delivery for combinations of soluble proteins or T4 displayed antigens with liposome and emulsion adjuvant formulations will be evaluated in parallel tracks, using the mouse model, for generation of protective antibody titers. The immunized mice will be challenged with (i) anthrax toxin, (ii) Sterne strain, and (iii) Ames strain, to determine the efficacy of the vaccines. The best combinations that induce protection in mice against challenge with virulent *Bacillus anthracis* will be tested in a guinea pig model. Immune responses will be characterized, and challenge experiments with the virulent anthrax strain will be performed to select the best vaccine product(s) that induce long-lasting immunity. The most promising anthrax vaccine candidates will be tested in a nonhuman primate model, in addition to characterization of immune responses, the immunized macaques will be challenged with the aerosolized spores of *Bacillus anthracis*. The duration of protection as well as pathological changes will be assessed.

Grant: 1U01AI056446-01
Program Director: HALL, ROBERT H.
Principal Investigator: HIGGINS, DARREN E PHD
Title: Novel Vaccine Strategy for *Listeria monocytogenes*
Institution: HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA
Project Period: 2003/08/01-2008/01/31

DESCRIPTION (provided by applicant): *Listeria monocytogenes* is an intracellular bacterial pathogen that causes serious foodborne illness in pregnant women, the elderly, infants and immunocompromised individuals. *L. monocytogenes* infections have the highest case fatality rate of all reported foodborne illnesses. *L. monocytogenes* can be easily cultured outside of host cells under standard laboratory conditions, making *L. monocytogenes* a significant public health risk and a significant potential threat as a biological weapons agent. Animal models show that protective immunity to *L. monocytogenes* is mediated by CD8⁺ effector cells that recognize and eliminate infected host cells. Vaccine studies have demonstrated that stimulation of protective CD8⁺ effector cells requires subclinical infection with live bacteria. However, immunization of humans with virulent bacteria fully capable of intracellular replication imposes a significant health risk to any population as a vaccine strategy, especially for those individuals inherently at risk to *L. monocytogenes* infection. We have recently developed a novel strategy for the generation of replication-deficient bacterial vaccine vectors that are capable of stimulating protective CD8⁺ effector cell responses. The focus of this proposal is to utilize this approach to produce non-replicating *L. monocytogenes* vaccine strains capable of generating protective CD8⁺ effector cell responses. In Specific Aim I, we will construct non-replicating *L. monocytogenes* vaccine vectors to deliver protective native bacterial antigens to the cytosol of professional and nonprofessional antigen presenting cells (APC) for endogenous processing and MHC Class I presentation. In Specific Aim II, we will determine the kinetics of antigen delivery to APC and the requirement of bacterial viability for efficient antigen delivery. In Specific Aim III, we will determine whether uptake of the vaccine constructs sensitizes APC for recognition by *L. monocytogenes*-specific effector cells. In Specific Aim IV, we will determine whether antigen specific effector cells are stimulated following immunization with the replication-deficient vaccine constructs, and assess stimulation of protective antilisterial immunity. Our goal is that following completion of the proposed studies, a safe and effective replication-deficient vaccine formulation will be identified that is suitable for clinical trials. It is also envisioned that these studies will provide a foundation for the development of replication-deficient vaccine vectors against other intracellular pathogens using a similar approach.

Grant: 1U01AI056452-01
Program Director: ZOU, LANLING
Principal Investigator: CLEMENTS, JOHN D
Title: Novel adjuvants for biodefense vaccines
Institution: TULANE UNIVERSITY OF LOUISIANA NEW ORLEANS, LA
Project Period: 2003/09/15-2008/02/29

DESCRIPTION (provided by applicant): An ideal vaccine against potential agents of biological warfare/bioterrorism should be safe, easy to deliver, provide long-lasting protection, require only one or a few doses, and provide protective immunity against different agents. Moreover, since mucosal and respiratory surfaces represent the first productive points of contact with the human host for many aerosolized biological agents, the role of mucosal immunity in protection needs to be examined. Nonparenteral delivery of vaccines (i.e., by the mucosal or transcutaneous route) has been shown to be effective at inducing both humoral and cellular antigen-specific immune responses in both the systemic and mucosal compartments of immunized animals and humans. Such "needle free" immunizations offer many potential advantages over parenteral immunization and are being evaluated by a number of groups for use in biodefense vaccines. Induction of immune responses by these nonparenteral routes is dependent upon the co-administration of appropriate adjuvants that can initiate and support the transition from innate to adaptive immunity. In this application we are proposing to investigate three novel adjuvants (LTR192G), CpG ODN, CTA1-DD) for their ability to induce high titer, long lived, protective antibody responses against relevant vaccine antigens from *B. anthracis* (rPA), *Y. pestis* (F1-V), botulinum toxin (Hc), and staphylococcal enterotoxin B (SEBv). The findings from these studies should be broadly applicable to other antigens from these and other biological agents. The primary objective of these studies is the optimization and preclinical testing of these novel adjuvants, each of which has previously shown promise in other infectious disease vaccines delivered mucosally or transcutaneously. It is expected that one or more of these adjuvants will be considered as candidates for future Phase I-II-III testing in clinical trial programs. Another objective of this application is to determine if nonparenteral boosting can induce a protective secondary immune response in animals that have been parenterally primed and if that response can be redirected to include a mucosal immune component. Challenge studies will allow us to correlate immune responses with protective efficacy and determine the contribution of mucosal immune responses to protection. Finally, we will determine the effectiveness of a combined vaccine consisting of relevant antigens from the four different pathogens with the specific objective of determining synergy or interference between the vaccine components and the role of adjuvants and route of delivery in overcoming interference.

Grant: 1U01AI056456-01
Program Director: TAYLOR, KATHERINE A.
Principal Investigator: STRAYER, DAVID S
Title: FOCUSING IMMUNITY vs BOTULINUM TOXIN WITH CYTOKINE DNA
Institution: THOMAS JEFFERSON UNIVERSITY PHILADELPHIA, PA
Project Period: 2003/09/01-2008/02/29

DESCRIPTION (provided by applicant): The toxin of *Clostridium botulinum*, particularly administered by inhalation, is a potential weapon of bioterrorism and germ warfare, and protecting people from the potentially devastating effects of its use is a high priority. At the same time, botulinum toxin (BT) has very important medicinal uses as an antispasmodic agent. We propose a collaborative project to use gene delivery of cDNAs encoding BT, serotype A (BT/A), antigens, to be given in tandem with cDNAs encoding cytokines that preferentially stimulate secretory IgA antibody (SIgA) responses. The goal of these studies is develop immunization regimens that protect from inhalational BT without precluding the important therapeutic uses of this agent. We will use both plasmid and recombinant adenoviral gene delivery vehicles to deliver both the heavy chain of BT (HC). Antibody elicited by HC protects from challenge with the complete toxin. These vectors will also deliver DNAs encoding cytokines that preferentially weight immune responses in favor of SIgA, at the expense of serum IgG antibody: transforming growth factor-beta1 and interleukins-4 and -5 (IL-4, IL-5). Our main hypothesis is: Immunizing regimens using cytokines that favor SIgA can elicit protective mucosal immunity to BT without system antibody. To test this hypothesis we propose four aims. We will: 1. Produce plasmid and adenovirus expression constructs to deliver BT/A-HC and cvtokine cDNAs. 2. Define parameters of local and svstemic immunity to BT, elicited by plasmid and rAd vectors. 3. Add cytokine-carrying vectors, individually and then in combinations, to these immunization regimens. 4. Modify expression constructs and administration regimens to optimize selectivity, protection, and safety. Thus, we will apply current understanding of the signaling mechanisms that occur during the activation of the secretory immune system to the need to protect people from the potential weaponization of botulinum toxin without limiting its important therapeutic applications.

Grant: 1U01AI056460-01

Program Director: ZOU, LANLING

Principal Investigator: MICHALEK, SUZANNE M PHD
MICROBIOLOGY:IMMUNOLOGY

Title: Development of a Mucosal Vaccine Against *F. tularensis*

Institution: UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL
BIRMINGHAM

Project Period: 2003/09/15-2008/02/29

DESCRIPTION (provided by applicant): Most infectious agents cause disease via our mucosal surfaces, which also applies to biological warfare agents. Therefore, in developing a vaccine against infectious/biological warfare agents, it is important to induce responses that would act at mucosal surfaces, as well as in the systemic compartment. *Francisella tularensis* is a gram-negative pathogen and cause of tularemia. This microorganism is a "category A pathogen and biological warfare agent". The overall goal of this project is to develop a safe mucosal vaccine effective in inducing protective responses against infection by *F. tularensis*. Specifically, we will 1) Determine the immunogenicity of heat shock proteins of *F. tularensis* and the effect of the saponin analog GPI-0100 and of ricin B in modulating host immune responses following systemic or intranasal immunization of mice. Serum and secretions will be collected and assayed for the nature and level of antigen-specific antibody activity by ELISA. Cells will be cultured and assessed for antigen-specific T cell proliferative responses and cytokine production (by ELISA). The effectiveness of the response on protection will be assessed following systemic or mucosal challenge with *F. tularensis*. 2) Determine the cellular mechanism(s) by which *F. tularensis* and its LPS, and the adjuvants modulate host responses. The role of Toll-like receptors (TLRs) and the B7 costimulatory system in mediating host responses and infection will be assessed in vitro and in vivo. Antigen-presenting cells from normal and TLR deficient mice will be stimulated in vitro and assessed for changes in the expression of MHC and B7 by flow cytometry and for cytokine production by ELISA. The cell signaling pathways involved in cell activation will also be determined. TLR- and B7-knockout mice will be used to determine the role of TLRs and B7 in responses to *F. tularensis* and its LPS. Humoral and cellular responses will be assessed as indicated above. Clearance of *F. tularensis* will be measured by microbiologic analysis. 3) Derive and characterize genetically defined attenuated strains of *F. tularensis* LVS with mutations in the shikimate and/or purine metabolic pathways for use as a live vaccine. Mutants will be derived and tested in mice for their safety, persistence in host tissue by microbiologic analysis, immunogenicity by inducing cellular (cell proliferation and cytokine production) and humoral (nature and level of antibody activity by ELISA) responses, and effectiveness in inducing protective immunity. These studies will define the role of the innate and adaptive immune systems in inducing protective responses to *F. tularensis* and will define a safe and efficacious vaccine against mucosal or systemic challenge with *F. tularensis*.

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Grant: 1U01AI056476-01
Program Director: KORPELA, JUKKA K.
Principal Investigator: DAVID, SUNIL A MD
Title: Gram-Negative Sepsis: Pharmacophore-Based Therapeutics
Institution: UNIVERSITY OF KANSAS LAWRENCE LAWRENCE, KS
Project Period: 2003/08/01-2008/01/31

DESCRIPTION (provided by applicant): Gram-negative sepsis, a common and serious sequel of systemic bacterial infections is the leading cause of mortality, accounting for some 200,000 fatalities annually in the US alone. The pathogenesis of Gram-negative septic shock is due to the host response to endotoxins, or lipopolysaccharides (LPS), present on the surface of gram-negative bacteria. There are, to date, no FDA-approved therapeutic options targeting the endotoxin itself to prevent or treat this disease. We have shown that relatively simple, and synthetically easily accessible molecules of the lipopolyamine class specifically bind to LPS and neutralize its toxicity both in vitro and in animal models of septic shock. The affinity of the lipopolyamines toward LPS, however, is relatively weak (2-10 M). In this proposal, our goal is to identify high-affinity LPS binders with nonlipopolyamine scaffolds as novel leads for the therapy of Gram-negative sepsis. A focused library of ~6000 compounds, each possessing the primary pharmacophore for LPS binding will be screened using a well-established fluorescence displacement method implemented in HTS formats. Binding, however, does not necessarily manifest in neutralization of LPS toxicity. For neutralization, an additional, appropriately positioned long-chain aliphatic group is essential. High-affinity binders ("hits") identified in HTS will be alkylated appropriately to generate LPS-neutralizing compounds (sequestrants). In in vitro assays, the potency of lead compounds in inhibiting the release of LPS-mediated proinflammatory cytokines such as tumor necrosis factor will be characterized. In a select subset of promising leads identified in the screens described above, we will verify that the mechanism of action of inhibition of LPS toxicity is via its sequestration by showing that relevant upstream cell-signaling events are blocked. The protective effects of particularly promising molecules will then be examined in two well-established murine models of gram-negative sepsis. We will systematically evaluate the toxicity of the test-compounds in a carefully chosen panel of in vitro assays. Molecular modeling techniques will be applied in an effort to correlate experimentally observed binding affinities of the test compounds with features of molecular interaction such as binding geometry, H-bonds, electrostatic, hydrophobic, and van der Waals contributions to the free energy of binding. Based on the data from the primary screen, in silico modeling, and biological assays, we will synthesize a series of analogues around promising leads using a combination of focused virtual library screening and classical medicinal chemistry approaches.

Grant: 1U01AI056477-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: BROUILLETTE, WAYNE J PHD
Title: A Novel Target for New Anti-Anthrax Drugs
Institution: UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL
BIRMINGHAM
Project Period: 2003/08/01-2008/01/31

DESCRIPTION (provided by applicant): The use of *Bacillus anthracis* in acts of terrorism and/or biological warfare is a demonstrated threat to U.S. security. The long-term objective of this proposal is to develop inhibitors against a new drug target, the anthracis enzyme nicotinamide adenine dinucleotide (NAD) synthetase, that are effective therapeutic agents for preventing and/or treating infections caused by *B. anthracis*. NAD synthetase catalyzes the last step in both the de novo and salvage pathways for the biosynthesis of NAD, an essential cofactor in energy metabolism. Since exogenous NAD cannot support bacterial growth due to insufficient cell membrane permeability/transport, inhibitors of NAD biosynthesis should be bacteriostatic and/or bacteriocidal. We have identified the first low micromolar inhibitors of *Bacillus* NAD synthetase, and these effectively inhibit the vegetative growth of *Bacillus anthracis*, and are bacteriocidal, at concentrations around 1 (μ g/mL. We will now perform reiterative design, chemical synthesis, and in vitro analysis to develop mature lead compounds. The Specific Aims are: (1) Using parallel, solution phase synthetic chemistry, we will optimize inhibitory activities for existing classes of lead structures until low nanomolar inhibitors are obtained. (2) Determine the molecular mechanism by which existing lead synthetic compounds inhibit NAD synthetase. In addition to protein crystallography, we will: (a) Measure the K_i and evaluate the type of inhibition for synthetic inhibitors of NAD synthetase. (b) Characterize the enzyme homodimer/monomer equilibrium and modulation by inhibitors. (c) Perform photoaffinity labeling experiments using inhibitors containing a photoreactive alkylating group (azide). (3) Alternative structural templates will be developed as new NAD synthetase inhibitors. All synthetic compounds will be evaluated in high throughput screens as enzyme inhibitors (IC_{50} and/or K_i) and antibacterials (MIC). Selectivity for the inhibition of *Bacillus* NAD synthetase over the human (erythrocyte) enzyme will also be evaluated.

Grant: 1U01AI056485-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: SWEETNAM, PAUL M PHD
Title: Novel Antibiotics against Gram-Negative Bacteria
Institution: SURFACE LOGIX, INC. BRIGHTON, MA
Project Period: 2003/08/15-2005/07/31

DESCRIPTION (provided by applicant): Gram-negative bacteria are a major cause of natural outbreaks of disease and feature prominently in the biological arsenals of rogue nations. Compounding this problem is the fact that bacteria are becoming increasingly resistant to current therapeutics, reducing the ability of today's antibiotics to treat the victims of natural outbreaks or attacks from engineered pathogens. We propose to target LpxK, an essential kinase involved in the biosynthetic pathway of Lipid A, a major constituent of the gram-negative outer membrane, as a means to develop novel compounds that can prevent or treat bacterial infections with gram-negative bacteria. Innovative surface-based assays will be used to screen compounds against LpxK. Once promising lead compounds are identified, we will perform iterative rounds of medicinal chemistry, optimizing their ability to block enzymatic modification, their efficacy on a variety of bacteria and their pharmacokinetic properties.

Grant: 1U01AI056487-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: DOW, STEVEN W BA
Title: Antigen Presentation and Pulmonary Immunity to Plague
Institution: COLORADO STATE UNIVERSITY-FORT FORT COLLINS, CO
COLLINS
Project Period: 2003/08/15-2007/01/31

DESCRIPTION (provided by applicant): The overall goal of this project is to develop a novel mucosal vaccine for generating rapid and effective pulmonary immunity against aerosolized *Yersinia pestis*. These studies will focus on a novel vaccine delivery system that consists of liposome-antigen-nucleic acid complexes (LANAC). Our preliminary studies indicate that LANAC vaccines can elicit marked and long-lived T cell responses against both peptide and protein antigens, with an efficiency that in most cases exceeds that elicited by viral-vectored or dendritic cell vaccines. Moreover, preliminary data also indicate that LANAC vaccines can elicit marked intrapulmonary immunity and humoral immunity and are effective after mucosal administration. The ability to elicit strong cellular immunity may be particularly useful for control of *Yersinia* within infected macrophages. Therefore, the objectives of this proposal are to define the immunological mechanisms by which liposome-nucleic acid complexes enhance antigen presentation and to determine whether mucosal LANAC vaccines formulated with either *Yersinia* protein or peptide antigens can elicit protective immunity against aerosol challenge with *Yersinia*. The specific aims of this project are to (1) determine how liposomes and nucleic acids interact to enhance antigen presentation; (2) identify critical antigen-presenting cell targets for LANAC and how mucosal routes of immunization affect antigen presentation; (3) determine whether mucosal or parenteral vaccination with recombinant *Yersinia* F1 or V antigens can elicit protective immunity to aerosol challenge; (4) determine whether small secreted peptides from *Yersinia* can elicit protective CTL responses. These studies have relevance to the stated objectives of this RFA because they will yield important mechanistic information on a novel vaccine adjuvant for use in immunization against plague and other Category A-C agents. In addition, this project will provide critical proof-of-principal validation of the LANAC vaccine approach and the ability to elicit rapid pulmonary immunity against inhaled *Yersinia*, particularly after mucosal immunization.

Grant: 1U01AI056489-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: MODLIN, ROBERT L MD
Title: CD1-targeted Vaccination against Tuberculosis
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 2003/08/01-2008/01/31

DESCRIPTION (provided by applicant): The long-range goal of this project is to develop and validate the CD1-based vaccine technology platform to immunize humans against microbial pathogens. This approach is based on the knowledge that several of the CD1 proteins have specific targeting sequences that direct their traffic into endosomal compartments, structures also critical for antigen presentation by MHC class II molecules to CD4+ T cells. We will determine whether this novel vaccine strategy can induce protective immunity against Mycobacterium tuberculosis, a worldwide killer, and now because of multi-drug resistant tuberculosis, a potential agent of a bioterrorist attack. We propose to determine the trafficking pattern of CD1 chimeras in antigen presenting cells including monocytes and dendritic cells. The ability of CD1 targeting to be used in immunization in humans will be assessed by studying the immune response to an M. tuberculosis antigen ESAT-6. In addition, the ability of ESAT-6/CD1 fusion constructs to immunize human T cell responses in vitro will be investigated. To determine whether ESAT-6/CD1 chimeras can induce protective immunity, ESAT-6/CD1 fusion constructs will be used to immunize mice, which will be subsequently challenged with virulent M. tuberculosis. The studies proposed will help develop and test the efficacy of CD1-based DNA vaccines for the prevention of infectious disease, including those from natural pathogens and bioterrorist attacks. Specifically, it should be possible to develop a new approach to the prevention of tuberculosis, including multidrug resistant tuberculosis, in humans. Finally, the CD1-based vaccine technology should prove useful in the vaccination against microbial pathogens in which MHC class II-restricted presentation of antigen to CD4+ T cells is required for host defense.

Grant: 1U01AI056493-01
Program Director: TAYLOR, KATHERINE A.
Principal Investigator: MARKS, JAMES D MD
Title: Development of botulinum neurotoxin immunotherapy
Institution: UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA
Project Period: 2003/07/15-2007/12/31

DESCRIPTION (provided by applicant): Botulinum neurotoxins (BoNTs) are classified as one of the six highest-risk threat agents for bioterrorism (the 'Category A agents'). BoNTs have been produced and weaponized by rogue nations and deployed by terrorist groups. The overall aim of this application is to generate neutralizing human compatible monoclonal antibodies (mAbs) to the BoNTs for prevention and treatment of botulism resulting from intentional exposure to toxin. Achievement of this goal requires three components: 1) determination of the extent of BoNT gene diversity of the seven toxin serotypes (A-G); 2) generation of panels of broadly neutralizing human compatible mAbs to the seven BoNT serotypes; and 3) in vitro and in vivo characterization of mAbs with respect to serotype cross reactivity and neutralization. These goals will be achieved by an inter-institutional team of botulism experts who have worked in this field for more than ten years: Eric Johnson, BoNT genetics and toxin production; James D. Marks, antibody engineering; and Leonard Smith, BoNT vaccine development and animal models of toxin neutralization. They will be assisted by one of the pioneers of microbial genetic characterization, Paul J. Jackson. The extent of toxin genetic diversity will be determined by establishment of a large repository of geographically disperse Clostridial strains that produce BoNTs. Strains will be characterized by pulsed field gel electrophoresis and amplified fragment length polymorphism (AFLP). These results will guide sequencing of selected BoNT genes to determine the extent of toxin diversity within and between serotypes. Based on these results, BoNT's and recombinant BoNT fragments will be expressed and purified and used as immunogens for mAb generation. Phage antibody libraries will be constructed from the V-genes of immunized mice, humans, and mice transgenic for the human immunoglobulin locus. High affinity, potentially neutralizing human compatible (human or humanized) antibodies to each of the BoNT serotypes will be selected from these libraries. Where necessary, murine antibodies will be humanized and mAb affinity increased by molecular evolution to achieve potent toxin neutralization. It is anticipated that at the end of the five year project period, a panel of mAbs will have been generated which in the relevant animal models broadly neutralize each of the seven BoNT serotypes. All antibodies will be human compatible (either humanized or fully human in sequence) and will be ready for transfer to a manufacturing facility for cGMP production, toxicology studies, and human testing. It is also anticipated that the project will define the range of BoNT diversity and determine how this diversity affects immunogenicity and antibody recognition. Such information will be invaluable for vaccine development as well as diagnostic testing and microbial forensics.

Grant: 1U01AI056513-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: GRANDI, GUIDO PHD
Title: Novel vaccine candidates for Y. pestis from genomics
Institution: CHIRON S.P.A. SIENA,
Project Period: 2003/08/01-2006/01/31

DESCRIPTION (provided by applicant): This proposal aims to use a genome wide screen of the entire Y. pestis complement of proteins to identify novel protective antigens for inclusion in a multivalent vaccine designed to protect military personnel and the civilian community against possible terrorist attacks with weaponized plague bacteria. Of the more than 4000 predicted protein coding genes identified by complete genome sequencing, approximately 476 are predicted to be secreted and/or associated with bacterial membrane and are, as such, potentially protective antigens. We propose to clone the genes for each of these proteins, to express each as a recombinant protein in Escherichia coli and, using antibodies raised in mice against these antigens, definitively determine their cellular location by ELISA and flow cytometry on intact bacteria. Complete genome hybridizations will be used to identify, among these potential candidates, genes common to the majority of clinical isolates of Y. pestis. In addition, the candidate list will be further refined by analysis of gene expression in vitro and in vivo using DNA chip technology. Finally, the capacity of the candidate antigens to induce immunologic protection will be assessed by in vivo challenge experiments in mice and by assays of serum bactericidal activity in vitro. We expect to identify several novel antigens capable of conferring protection to mice against aerosol challenge with virulent Y. pestis bacteria. The approach of using modern genomic technologies in vaccine design has been pioneered in our laboratories and has been termed "reverse vaccinology". The approach has been remarkably successful in the identification of novel antigens that induce protection against Neisseria meningitidis, some of which are included in a vaccine currently undergoing clinical evaluation.

Grant: 1U01AI056514-01
Program Director: TAYLOR, KATHERINE A.
Principal Investigator: HENDERSON, IAN PHD
Title: Fast-Track Production of a Heptavalent Botulinum Vaccine
Institution: DYNPORT VACCINE COMPANY, LLC FREDERICK, MD
Project Period: 2003/09/01-2008/02/29

DESCRIPTION (provided by applicant): Botulinum neurotoxin, an NIAID class A agent, has been weaponized, thus dictating an urgent need for a safe, efficacious vaccine that will protect against all seven known serotypes of the toxin. The two specific aims of this project are first, the fast-track development (within one-to-two years) of a safe and efficacious pentavalent vaccine for the neurotoxin that will provide immunologic protection against toxin serotypes A, B, C, E and F; and second, development of a fully protective heptavalent vaccine to include serotypes D and G within five years. The partners for this project include representatives from industry (DynPort Vaccine Company LLC:DVC), academe (University of Nebraska-Lincoln: UNL and the University of Colorado Health Sciences Center: UC-HSC) and the military (U.S. Army Medical Research Institute of Infectious Diseases: USAMRIID). The approach we will take to achieve these aims is based upon the successful development and expression in the yeast, *P. pastoris*, of serotype-specific toxin heavy-chain protein fragments, that, when purified and administered to experimental animals, provide protective immunity against challenge with lethal doses of the botulinum neurotoxins. Monovalent vaccine candidates against serotypes A and B are already in current Good Manufacturing Practices pilot production, and vaccine candidates for serotypes C, E and F are in advanced stages of process development at UNL, thus facilitating the likelihood of success of the first specific aim. The methodologies established for development of vaccine candidates against serotypes A, B, C, E and F provide a rich body of knowledge on which the development of equivalent vaccine candidates for the remaining two serotypes (D and G) will be based, thus facilitating accomplishment of the second specific aim. UC-HSC will contribute expertise in formulating and stabilizing both liquid and lyophilized vaccines, essential for the achievement of both specific aims. The proposed research will have a high probability of success, in part, because of the highly innovative approach of having the industry partner, DVC, whose business model is vaccine development through management and coordination of scientific subcontractors, serve as the lead institution. The impact of the successful completion of the project will be an effective heptavalent vaccine for prophylactic immune defense against botulinum neurotoxin within an expedited, but realistic, timeframe and elimination of botulinum toxin as a weapon of mass destruction.

Grant: 1U01AI056516-01
Program Director: TAYLOR, KATHERINE A.
Principal Investigator: MORRISON, SHERIE L PHD BIOLOGY NEC:BIOL
NEC-UNSPEC
Title: Immunotherapeutics to Prevent & Treat BoNT Intoxication
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 2003/07/15-2007/12/31

DESCRIPTION (provided by applicant): Among agents identified as potential weapons under CDC category A, or high priority agents, is botulinum toxin (BoNT). BoNT poses a major bioweapon threat because of its extreme potency and lethality, its ease of production and transport, as well as the need for prolonged intensive care among affected persons. The goal of this proposal is to develop immune-based therapeutics to counter the use of BoNT as a weapon of bioterrorism. Since BoNT cannot cross intact skin, when used as a bioweapon, it is expected that BoNT would gain entry via epithelial cells of the gastrointestinal (GI), respiratory tract or both. Experiments are proposed to determine what sequences are required for BoNT transport by human epithelial cells. We will first determine the minimal toxin fragment required for uptake across polarized human epithelial cells. Both binding and transcytosis will be measured. Site-directed mutation will be used to further map the site on the toxin required for binding, transcytosis or both. Polarized epithelial cell lines originating from both the lung and the GI tract will be used to determine if access across different epithelia requires the same toxin domain. Monoclonal antibodies (mAbs) will be produced to the domain (or sub-domain) responsible for epithelial binding and transcytosis. The mAbs will be tested for their ability to inhibit binding and/or transcytosis. Both the intracellular trafficking pathway and the receptor(s) on the epithelium used for transcytosis will be identified. If a protein functions in the receptor, it will be cloned. The role of antibody isotype and valence in the inhibition of BoNT transport across the epithelium will be determined. Variable regions from mAbs will be cloned and expressed as IgA, which is polymeric and hence exhibits enhanced avidity, and as secretory IgA (sIgA) with covalently attached secretory piece, which is also polymeric but is more resistant to proteases. The role of effector function activation and valence in the context of IgG will also be investigated. Efforts will be made to produce an antibody-based protein that can gain access to the cytosol of the neuron and neutralize intracellular BoNT. mAbs effective in neutralizing the catalytic activity of BoNT will be produced. We will attempt to deliver these into the cytoplasm of neurons either by genetically fusing them to antibodies that recognize surface molecules of neurons and are endocytosed or by replacing the catalytic domain of BoNT with a scFv version of the neutralizing mAb.

Grant: 1U01AI056536-01
Program Director: ZOU, LANLING
Principal Investigator: LU, SHAN PHD
Title: Multi-gene plague vaccine with expanded protection
Institution: UNIV OF MASSACHUSETTS MED SCH WORCESTER, MA
WORCESTER
Project Period: 2003/09/30-2005/08/31

DESCRIPTION (provided by applicant): The overall objective of this application is to rapidly develop much needed alternative plague vaccine candidate formulations based on our recent discovery that a modified *Y. pestis* V antigen (LcrV) based DNA vaccine was highly effective in protecting experimental mice against lethal intranasal challenge with *Y. pestis*. Our preliminary data suggested that good protection resulted from improved antigen presentation and possibly through improved Th1 type immune responses. This is the first time a DNA-based plague vaccine induced complete protection against lethal mucosal challenge in any animal model. We now propose to conduct vigorous validation and testing in the first 18-24 months to develop a subunit plague vaccine formulation using this novel modified V antigen as the core protective component. The protection efficacy of this subunit vaccine, delivered in the forms of DNA, protein, or a combination of both, will be evaluated against lethal *Y. pestis* challenge via the airway mucosa. Additional immunological studies will be conducted to confirm the improved efficacy of this modified V antigen. We also propose to continue our search for additional protective *Y. pestis* antigens to be incorporated into the above primary formulation so that a true multi-gene plague vaccine can be developed to achieve broader protection to discourage attempts to engineer vaccine resistant *Y. pestis* strains. This search will focus primarily on antigens involved in the function of the *Y. pestis* Type III secretion apparatus including YopB, YopD and YscF. A variety of combination formulations will be tested among these candidates and the modified V antigen to identify the formulation that can provide the most effective protection. This project will employ the most advanced DNA immunization technology in combination with small-scale protein production to quickly develop potential subunit-based plague vaccine products.

Grant: 1U01AI056546-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: MOGRIDGE, JEREMY S PHD
Title: Development and testing of anthrax toxin inhibitors
Institution: UNIVERSITY OF TORONTO TORONTO ONTARIO M5s 1A
ON
Project Period: 2003/09/30-2008/03/31

DESCRIPTION (provided by applicant): The objective of our research proposal is to develop inhibitors of anthrax toxin that prevent the death of animals challenged with *Bacillus anthracis* spores. Anthrax toxin is a combination of three non-toxic proteins that are secreted separately by the bacterium and then assemble into toxic complexes on the mammalian cell surface. The protective antigen component of the toxin binds the anthrax toxin receptor and oligomerizes into heptamers that bind the toxic enzymes, edema factor and lethal factor. Inhibitors of anthrax toxin that block either toxin assembly or cytosolic delivery of the enzymatic proteins are expected to be effective anthrax therapeutics because the toxin is necessary for disease progression and causes death of the patient. We have previously synthesized a molecule consisting of multiple copies of a toxin-binding peptide coupled to a polymer backbone. The peptide alone can prevent the assembly of the tripartite toxin in vitro and its polyvalent display by the backbone increases its effective activity. We demonstrated that this inhibitor prevents the activity of anthrax toxin in rats, indicating that this molecule is a promising lead compound for an anthrax therapeutic. During the period of this proposal, we will test whether this inhibitor can prevent death of mice challenged with *Bacillus anthracis* spores. We will also assess the inhibitor's toxicity and pharmacokinetics. In addition to testing this lead compound, we will synthesize and test derivatives to develop a mature compound. The first class of molecules we synthesize will display inhibitory peptides from a variety of polymeric backbones and nanoparticles, which will be chosen for properties such as biocompatibility and bioavailability. The second class of molecules will consist of backbones of defined molecular weight that display multiple copies of the inhibitory peptide. We will also optimize the sequence of the peptide to increase its inhibitory activity. Iterative design and pharmacological testing will facilitate the development of inhibitors with high potency, long lifetime, and low toxicity. These inhibitors may be an effective adjunct to antibiotic therapy in the treatment of anthrax.

Grant: 1U01AI056559-01
Program Director: ZOU, LANLING
Principal Investigator: VAN NEST, GARY A PHD
Title: Advanced anthrax vaccine made with ISS DNA formulations
Institution: DYNAVAX TECHNOLOGIES CORPORATION BERKELEY, CA
Project Period: 2003/07/15-2006/12/31

DESCRIPTION (provided by applicant): We propose to use ISS to develop an improved anthrax vaccine that will be safe and will provide immunity after one or two immunizations. This vaccine will be comprised of recombinant anthrax protective antigen (rPA) delivered with an immunostimulatory (ISS) oligonucleotide adjuvant enhanced by a novel coacervate formulation. We will compare the ISS sequence currently in clinical trials, 1018, with our more active ISS sequence, C274 to identify the most potent vaccine formulation. The use of ISS in the novel coacervate carrier should provide dramatic enhancement of both the speed and magnitude of the antibody response developed against rPA compared to current second generation approaches using rPA adsorbed to alum or compared to the currently licensed vaccine, AVA. The specific aims for this project are: 1. To test the immunogenicity of recombinant anthrax protective antigen (rPA) with and without combination with ISS coacervate formulations in mice, rabbits, and rhesus macaques; sera will be evaluated by PA-specific ELISAs and for the capacity to neutralize the anthrax toxins using in vitro cell culture assays. 2. To establish the ability of 1-2 doses of the optimized ISS coacervate/rPA vaccine to protect experimental animals against anthrax in vivo following challenge with *Bacillus anthracis* spores. A. Protection of mice against challenge with *B. anthracis* spores administered by the intranasal route. B. Protection of rabbits against inhalation anthrax following aerosol challenge with *B. anthracis* spores. C. Protection of rhesus macaques against inhalation anthrax following challenge with *B. anthracis* spores. 3. To perform toxicological evaluation and to initiate GMP production of the ISS coacervate/rPA vaccine, assuming positive results in Specific Aims 1-3. Because of the high cost of the later Specific Aims (Aims 3C and 4), activities for each Specific Aim will be performed sequentially, and feasibility will be established before the project moves onto the next Aim. Assuming success in the individual Specific Aims, this proposal is intended to produce an IND-ready new anthrax vaccine that could rapidly move into clinical testing.

Grant: 1U01AI056580-01
Program Director: ZOU, LANLING
Principal Investigator: CEASE, KEMP B MD
Title: Molecularly Targeted Vaccines for Anthrax
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2003/08/15-2008/01/31

DESCRIPTION (provided by applicant): Inhalation anthrax has emerged as a significant and continuing biowarfare and bioterrorism threat. Though vaccine strategies have substantially controlled this disease in animal populations, the current vaccine licensed for human use has several shortcomings, including significant adverse reactions and low immunogenicity resulting in the need for multiple immunizations. The biology of anthrax infection and specifically the actions of its toxins have been elegantly elucidated at the molecular and cellular level. This presents a unique opportunity to rationally design and develop an improved vaccine that can counter current anthrax, as well as modified forms that may be encountered in the future. In this project, we will use the substantial body of knowledge and insight available relating to anthrax toxin to develop molecularly targeted vaccines to prevent the catastrophic effects of toxin following infection. Based on careful analysis of the crystal structure, sequence, and functional studies of all components of the anthrax toxins, we will develop soluble protein vaccines that direct antibody responses towards the critical protein segments involved in toxin activation, assembly, and adherence to cells. We will test these vaccine constructs for their ability to elicit strong antibody responses with the ability to neutralize toxin activity in in vitro assays. Based on analysis of responses to the soluble protein constructs, selected constructs will be moved into the adeno-associated virus vaccine platform for development of an expression vaccine. Traditionally such vaccines have exhibited higher immunogenicity, and AAV in particular has yielded very durable responses. The AAV-based vaccines will be tested for immunogenicity and their ability to elicit toxin-neutralizing antibodies, as well as for use in concert with soluble protein immunogens. Finally, the most promising constructs of both soluble protein and expressed forms will be tested in anthrax spore inhalation challenge studies in rabbits. Such experiments have been extensively validated against primate experiments previously and also appear to correlate most closely with human anthrax disease and protection. Through this approach, we will develop promising candidate anthrax vaccines that can protect against currently existing strains of anthrax, as well as new and enhanced threats based on engineered forms of anthrax.

Grant: 1U01AI056689-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: ALLAND, DAVID MD CLINICAL MEDICAL SCIENCES, OTHER
Title: Detection of Select Agents in Single-Well Assays
Institution: UNIV OF MED/DENT NJ NEWARK NEWARK, NJ
Project Period: 2003/08/01-2008/01/31

DESCRIPTION (provided by applicant): The emerging threat of bioterrorism has created a need for rapid diagnostic assays of pathogenic bacteria. To be maximally effective, assays must not only be rapid, sensitive, and specific, but also sufficiently simple and robust to be used in local healthcare settings, as well as in regional laboratories. Assays must detect all bacteria that are classified as select agents, and should also have the capacity to identify common infectious diseases. This latter feature will encourage both familiarity with the assays and their widespread distribution, assuring their availability if a bioterrorist attack occurs. We propose the development of novel approaches to real-time PCR that vastly expand the number of different pathogens that can be detected in a single assay well. These assays will have the same sensitivity and robustness as the current generation of real-time polymerase chain reaction (PCR) methods, but will have a dramatically expanded ability to distinguish different pathogen-specific nucleic acid sequences. Our laboratories have pioneered molecular beacons as sequence-specific hybridization probes for use in real-time PCR assays. Here, we propose the development of a new paradigm for the use of molecular beacons that will enable highly multiplexed detection. We will replace specific molecular beacons with mixtures of molecular beacons that act in concert. Sequences will be identified by detecting "fluorescence signatures" generated by multiple probes that hybridize to DNA sequences in characteristic fashions. The switch from single-probe identification to multi-probe, pattern-based identification will enable us to develop highly multiplexed assays using only a small number of probes or colors. We propose two related approaches. We will create a "sloppy" molecular beacon assay that will use six differently colored molecular beacons to distinguish among 60 or more different target sequences. We will also develop a "color-triplet coding" format that will enable unique labeling of as many as 56 different molecular beacons in the same assay well, utilizing only eight differently colored fluorophores. A distinguishing feature of these approaches is that they are based on real-time PCR technology, which has already been reduced to practice in commercial assays. Our specific aims: 1. To develop sets of "universal" PCR primers that amplify species-specific DNA sequences from all select bacterial agents and common bacterial pathogens. 2. To develop PCR assays that are able to distinguish over 60 different species-specific DNA sequences in a single assay well, utilizing only five differently colored, semi-specific "sloppy" molecular beacons. 3. To develop PCR assays that utilize "color-triplet coding" to uniquely label as many as 56 different species-specific molecular beacons.

Grant: 1U01AI057232-01
Program Director: GIOVANNI, MARIA Y.
Principal Investigator: BOCHNER, BARRY R PHD
Title: Detection of Bioterrorism Agents with PM Technology
Institution: BIOLOG, INC. HAYWARD, CA
Project Period: 2003/08/01-2007/01/31

DESCRIPTION (provided by applicant): Laboratories doing routine microbiology now must have tools that allow them to recognize and identify dangerous pathogens that could appear as part of their normal workflow. They need this capability to identify incipient bioterrorism attacks, to save lives of stricken people by rapid diagnosis, and to prevent the laboratory workers themselves from dangerous and uncontrolled exposures. Our proposal provides novel yet practical solutions to the real problems faced by microbiologists right now. Biolog will develop a highly advanced, bacterial identification system that can be used by any clinical microbiology laboratory to identify over 800 different bacterial species including bioterrorism microorganisms that could be used in an attack against humans or U.S. agriculture. This development is enabled by Biolog's newly developed, proprietary platform technology, Phenotype MicroArrays (PMs). PMs constitute a new genomic scale technology that gives unprecedented detailed information about the properties of live cells allowing scientists to test 2000 properties of a microbial cell simultaneously in a proprietary recording instrument called the OmniLog. The core technology is an image analysis system that detects changes in respiration of cells grown under different conditions. Because a large number of parameters are measured, an information-rich colorimetric pattern is generated for each bacterium, constituting a fingerprint of that bacterium. By testing a broad and representative collection of bacterial species in an automated high-throughput program, we will rapidly build a detailed database on phenotypic properties common to each bacterial species as well as properties that distinguish each species from its closest relatives. The database will be used to generate test panels capable of identifying over 800 species of both gram-negative and gram-positive bacteria in a single test. This will improve the simplicity, speed, and accuracy of bacterial identification far beyond the technology available to medical labs at this time. We will also develop new, highly selective and specific enrichment and culture media that will allow investigators to monitor for bioterrorism microorganisms in the environment using standard, inexpensive, low tech agar plates.

Grant: 1U01AI057276-01
Program Director: ZOU, LANLING
Principal Investigator: MARKHAM, PENELOPE N PHD
Title: PTE-based drug for antibiotic-resistant anthrax
Institution: INFLUX, INC. CHICAGO, IL
Project Period: 2003/09/01-2003/12/31

DESCRIPTION (provided by applicant): One of the major concerns about possible future bioterrorism attacks involving anthrax is that terrorist entities may easily develop B. anthracis strains resistant to many, if not all, currently recommended antibiotics. Our company has identified a promising class of synthetic compounds, pyridinium thio ethers (PTEs) that display strong bactericidal activity against a variety of Gram-positive bacteria, including B. anthracis. Some PTE compounds are active even against Bacillus spores. PTE compounds display little toxicity towards human cells in vitro, and are highly promising from the drug development standpoint: they are water-soluble, retain activity in the presence of human serum and are readily amenable to chemical modification and lead optimization efforts. Most importantly, it appears very difficult, if not impossible, to select Gram positive bacteria resistant to this class of compounds, which addresses the major shortcoming of the existing antibiotics as anti-anthrax drugs. Based on our preliminary data, we propose to develop PTE compounds as a narrow spectrum antibiotic for the post-exposure treatment of inhalation anthrax. In this proposal we seek funding for further biological characterization, chemical improvement and preclinical development of PTEs. The following specific aims will be pursued: AIM 1, Chemical improvement of PTE compounds for activity against B. anthracis, including synthesis of a diverse chemical library of PTEs and a QSAR-based iterative lead optimization; AIM2, Evaluation of in vitro activity of synthesized PTEs against B. anthracis, selection for resistance, and analysis of their toxicity to human cells; AIM 3, Determination of the biological mechanism of action of PTEs using genetic and biochemical approaches; AIM 4, In vivo anti-anthrax efficacy studies in a murine inhalational challenge model, including development of an oral formulation, acute toxicity tests, and preliminary pharmacokinetics; AIM 5, Pre-clinical (Pre-IND) drug development studies, including toxicity and pharmacokinetic studies with GLP material direc.

Grant: 1U01AI057286-01
Program Director: KOSHY, RAJEN
Principal Investigator: CHULAY, JEFFREY D MD
Title: Alphavirus Replicon Vaccines against Botulinum Neurotox*
Institution: ALPHAVAX HUMAN VACCINES, INC. RES TRIANGLE PK, NC
Project Period: 2003/09/01-2008/02/29

DESCRIPTION (provided by applicant): The objectives of the proposed research are to construct alphavirus replicon particle vaccines directed against all seven serotypes of botulinum neurotoxin (BoNT); perform in vitro evaluations and preclinical animal studies of these vaccines; perform production scale-up and GMP manufacture of a bivalent vaccine against BoNT (serotypes A and B), prepare and submit an Investigational New Drug (IND) application, and perform a phase I clinical trial. BoNT is highly lethal by the aerosol route and is a credible and significant bioterrorist threat. Existing investigational vaccines have significant shortcomings. We propose a novel approach, using a propagation-defective, single cycle, RNA replicon vector system, derived from an attenuated strain of Venezuelan equine encephalitis (VEE) virus, to produce virus-like replicon particles (VRP) expressing the carboxy-terminal half of the heavy chain (Hc) of all serotypes of BoNT. Preliminary animal studies have demonstrated the safety and feasibility of this approach, as both monovalent and bivalent VRP vaccines induced neutralizing antibody responses against the expressed BoNT Hc that conferred long-term protection against challenge with homologous toxin. The project will include seven specific aims. (1) Construct VRP vaccines expressing Hc fragments of all seven BoNT serotypes. (2) Perform in vitro characterization of these vaccines. (3) Evaluate preclinical safety, immunogenicity and efficacy in murine challenge models. (4) Perform scale-up development of processes for GMP-compliant manufacture of a bivalent (serotypes A and B) BoNT Hc fragment VRP vaccine selected for clinical evaluation. (5) Perform GMP manufacture of this vaccine. (6) Perform benchmark preclinical studies and prepare an IND. (7) Conduct a phase 1 clinical trial. To achieve these aims, human codon-optimized genes for BoNT Hc will be inserted into VEE replicon vectors. Using a novel DNA helper system that precludes replicon/helper recombination, the replicon RNAs will be packaged into VRP with a VEE glycoprotein coat that confers dendritic cell tropism. Safety, immunogenicity and efficacy will be evaluated using established animal models. Process scale-up, GMP manufacture, preclinical and clinical testing will be performed based on previous experience with a VRP vaccine for HIV. Results of these studies will provide the basis for future clinical trials of a comprehensive 7-valent BoNT Hc fragment VRP vaccine.

Grant: 1U01AI057291-01
Program Director: ZOU, LANLING
Principal Investigator: KAPLAN, NACHUM
Title: Novel antibacterial agents for treatment of Tularemia
Institution: AFFINIUM PHARMACEUTICALS, INC. Toronto, ON
Project Period: 2003/09/30-2005/08/31

DESCRIPTION (provided by applicant): A series of enoyI-ACP reductase (FabI) inhibitors have been identified with potent, narrow spectrum in vitro antibacterial activity against the agent of Tularemia, *Francisella tularensis*, as well as excellent in vitro potency against *Staphylococci*. Lead compounds have shown good in vivo activity in treating *Staphylococcus aureus* infections in rats and have good oral bioavailability. We propose experiments to assess the in vivo safety of the lead compounds, the frequencies and mechanisms of resistance development, and the efficacy of the lead compounds in animal models of *F. tularensis* infection. The experiments in this application aim to complete the preclinical characterization of the lead compounds, identify additional compounds with improved characteristics that retain excellent potency against *Staphylococci* and *Francisella*, and produce sufficient cGMP material to initiate Phase I clinical studies. We will determine the high-resolution protein structures of the *Staphylococcal* and *Francisella* FabI targets to guide the medicinal chemistry efforts. We aim to select a development candidate compound from this program that has excellent antimicrobial activity both in vitro and in vivo against *Staphylococci* and *Francisella* and that can be formulated for both IV and oral administration. Phase I and Phase II clinical trials are proposed in Years 4 and 5, as a narrow spectrum agent for the treatment of *Staphylococcal* infections. This funding will assure rapid progress of this new class of antibacterial agent through the last stages of discovery and preclinical studies and into the initiation of clinical trials. The funding will also assure that there are sufficient *F. tularensis* microbiological data and animal model data to warrant inclusion of the information on the product label as a narrow spectrum agent that could be used in the event of a *F. tularensis* release.

Grant: 1U01AI057315-01
Program Director: BEANAN, MAUREEN J.
Principal Investigator: DRMANAC, RADOJE PHD
Title: Comprehensive pathogen diagnostics with rSBH system
Institution: CALLIDA GENOMICS, INC. SUNNYVALE, CA
Project Period: 2003/09/29-2006/02/28

DESCRIPTION (provided by applicant): Development of sensitive, universal diagnostic tools for fast and complete analysis of pathogen DNA is a critical priority in the Nation's biodefense effort. This proposal describes an advanced rapid sequencing by hybridization (rSBH) system designed to detect and identify any pathogen at the single organism level in complex biological samples. Based on similar technology being developed at Callida Genomics for fast, low cost human genome sequencing, the pathogen rSBH system would allow military or public health personnel rapid, inexpensive, and comprehensive information on the nature of pathogen infection before symptoms develop. In a typical rSBH experiment DNA fragments extracted from samples are interrogated with universal sets of labeled probes in a sequence-specific hybridization/ligation assay. An ultra-sensitive CCD camera detects positive ligation events, generating sequence information that is assembled computationally to determine complete sequences of all DNA present in the sample. Because the rSBH probe set is universal, containing all probes of a given length, it can detect and determine complete sequence information for trace quantities of pathogen DNA present in complex biological samples. This represents a significant advance over other DNA-based diagnostics, which require sequence-specific probes. The specific aims of the project are to develop and demonstrate a prototype rSBH pathogen diagnostics product by sequencing complete genomes and diagnostic genes of *Yersinia pestis* and *Bacillus anthracis* from cell culture and blood samples.

Grant: 1U01AI058266-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: HU, LINDEN T MD
Title: Development of a vaccine for murine Lyme disease
Institution: NEW ENGLAND MEDICAL CENTER BOSTON, MA
HOSPITALS
Project Period: 2003/09/01-2007/02/28

DESCRIPTION (provided by applicant): The incidence and geographic distribution of Lyme disease in the U.S. has increased steadily since its first description in 1977. Efforts to stem the spread of the disease through controlling the population of its tick vector and/or the mouse reservoirs of the disease have met with only limited success. The only approved human vaccine to protect against Lyme disease was recently removed from the market by its manufacturer further highlighting the need for new approaches to controlling the disease. In this project, we propose the development of an orally- available vaccine targeted towards the mouse and tick reservoirs of the disease. The project is modeled on the highly successful oral rabies vaccine, Raboral, which uses a vaccinia virus (VV) vector to deliver its immunogen to wild foxes and raccoons. We will take advantage of the enormous amount of immunogenicity and safety data that has been generated for vaccinia virus in hopes of rapidly developing a release-able vaccine. The vaccine itself will consist of outer surface protein A (OspA) recombinantly expressed from a VV vector. OspA was the antigen used in the human vaccine. Extensive research has shown that it is immunogenic in mice, that mice vaccinated against OspA are protected against infection with *Borrelia burgdorferi* and that *B. burgdorferi* infected ticks feeding on mice vaccinated with OspA are sterilized of their infection and cannot transmit the disease to other animals. Prior attempts to use OspA to vaccinate wild animals have been hampered by the lack of an efficient, oral delivery system which is both stable under natural environmental conditions and can generate an intense immune response. The three aims of this project are to: 1) create a recombinant VV expressing OspA (W/OspA); 2) establish the kinetics and durability of the immune response to the recombinant W/OspA; and 3) test the efficacy of W/OspA administered orally in preventing transmission of *B. burgdorferi* to mice and in sterilizing infection in infected ticks.

Grant: 1U01AI060557-01
Program Director: VAN DE VERG, LILLIAN L.
Principal Investigator: CURTISS, ROY PHD
MICROBIOLOGY:MICROBIO
OGY-UNSPEC
Title: S. typhimurium Vaccine Against Bacterial Enteropathogens
Institution: WASHINGTON UNIVERSITY ST LOUIS, MO
Project Period: 2003/09/30-2008/03/31

Of the 18.9 million annual deaths (1997) due to infectious diseases, about 2 million are the result of infections by Salmonella and other related bacterial enteropathogens including Escherichia coli and Shigella species, and less closely related enteropathogens such as Vibrio cholerae, Campylobacter jejuni and Listeria monocytogenes. In addition, these bacteria are responsible for significant morbidity causing diarrheal and systemic diseases that can be transmitted to humans by contamination of food products and/or the water supply and such contamination can be willful. In the belief that improving health, nutrition and economic well-being (the latter dependent on the first two) provide the best means to enhance the quality of life globally and thus reduce conditions that result in warlike and terrorist behavior, we propose a vaccine developmental program based on our recent technical developments in using non-recombinant and recombinant attenuated Salmonella veterinary vaccines to prevent-reduce diarrheal diseases caused by bacterial enteropathogens. Our objectives include: (i) to further genetically modify a strain of Salmonella typhimurium that has been designed to minimize induction of immune responses to serotype-specific antigens and to maximize induction of cross protective immunity to common related antigens of S. enterica strains of diverse serotype and then fully evaluate this modified strain as a vaccine to reduce diarrheal diseases in humans caused by S. enterica serotypes and possibly by other bacterial enteric pathogens, especially Escherichia coli of the EPEC, ETEC and EHEC types and Shigella; (ii) to design, construct and fully evaluate an attenuated derivative of S. paratyphi A, with similar genetic attributes as the S. typhimurium vaccine designed for the same purpose, to induce cross protective immunity in humans to prevent enteric fever and to significantly reduce diarrheal diseases due to infection by diverse S. enterica serotypes and possibly by other bacterial enteric pathogens, especially E. coli of the EPEC, ETEC and EHEC types and Shigella; (iii) to further genetically modify the S. typhimurium and S. paratyphi A vaccines designed to induce cross protective immunity to also display biological containment so that they are less able to survive in the intestinal tract or in nature and/or die by lysis after approximately ten cell divisions following delivery to the immunized individual; and (iv) to design, construct and evaluate recombinant attenuated Salmonella vaccines, using optimal attributes for immunogenicity, biological containment and antigen delivery, to express antigens to further enhance induction of cross protective immunity to Salmonella-related bacterial enteropathogens or to confer protective immunity to one of the less Salmonella-related enteropathogens. We will also collaboratively work to develop our Master File, prepare and fully characterize candidate vaccine Master Seeds for stability and safety, prepare and submit protocols for IRB approvals, submit information necessary to obtain INDs, and perform any other work needed to arrange that the best candidate vaccines by clinically evaluated in human volunteers.

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Grant: 1U19AI056510-01

Program Director: BAKER, PHILLIP J.

Principal Investigator: FISCHETTI, VINCENT A PHD
MICROBIOLOGY:BACTERIOLOGY

Title: Pathogen-specific drug targets for weaponized bacteria

Institution: ROCKEFELLER UNIVERSITY NEW YORK, NY

Project Period: 2003/08/01-2008/01/31

DESCRIPTION (provided by applicant): The threat of engineered bacterial bioweapons that are resistant to current antibiotic treatments provides a powerful mandate to develop novel classes of therapeutic compounds. This proposal presents a concerted effort of three complementary groups at the Rockefeller University (Dr. Vincent Fischetti, Dr. Alexander Tomasz, and Dr. Erec Stebbins) to identify novel inhibitors of the Category A pathogens, *B. anthracis* and *Y. pestis*, as well as Category B food and water safety threats. The first and central research project is based on the observation that resistance to bacteriophage lytic enzymes, such as PlyG for *B. anthracis*, is an extremely rare event, from which we hypothesize that the binding domain of these enzymes targets a critical component in the bacterial cell wall to assure phage release (in a sense through evolution, the phage have performed the high throughput assay to identify this component). Thus, the pathway to the synthesis of this wall component represents a target for antibiotic development. The core project will therefore focus on the identification, isolation and characterization of the lytic enzyme binding substrate. Once identified, the pathway towards its synthesis will be determined. Those enzymes catalyzing the pathway will be isolated, purified and crystallized, and inhibitors identified. The cell wall structural experience of Alex Tomasz, feeds into the core project but also broadens it to a search for additional novel targets against *B. anthracis* by high resolution analysis of the cell walls and by the isolation of conditional lethal mutants - using in vivo transposition of a mariner type element. Cell walls occupy center stage in his project since (i) the target of PlyG is in the wall; (ii) cell walls play an important role in the devastating septic shock caused by *B. anthracis* infections and (iii) the genome of *B. anthracis* contains wall-related determinants that are virulence factors in other bacteria. Erec Stebbins will provide the expertise in protein structure and crystallization along with the identification of small molecule inhibitors for these structures. His project expands the novel therapeutics search to include *Y. pestis* and Category B pathogens through a translational component in computation drug discovery coupled to structural determination. In total, these efforts target central virulence factors in critical biowarfare pathogens.

Grant: 1U19AI056543-01
Program Director: SCHAEFER, MICHAEL R.
Principal Investigator: RICE, PETER A MD INTERNAL
MED:INFECTIOUS DISEASE
Title: Immuno-Prophylaxis-Therapy & Diagnosis of Tularemia
Institution: BOSTON MEDICAL CENTER BOSTON, MA
Project Period: 2003/09/15-2008/02/29

DESCRIPTION (provided by applicant): The major objectives of this Co-operative Research Program will be to: (1) develop a vaccine candidate(s) to protect against inhalational tularemia (caused by *Francisella tularensis*); (2) to develop a polyclonal antibody library to be used for passive immunization to ameliorate or prevent acute illness from *F. tularensis* acquired by the inhalational route and (3) to develop diagnostic systems to detect *F. tularensis* in clinical specimens and in the environment using immunochemical and/or gene amplification methods. In Project 1, we will prepare and use as vaccine candidates in experimental systems, *F. tularensis* lipopolysaccharide derived O-polysaccharide and capsular polysaccharides. We will use conjugates and clinically relevant adjuvant and delivery systems to recruit T cell help to enhance immune responses. We will also create peptide surrogates (called mimics) of the two saccharide prototypes and use these for immunization. Mice will be immunized to assess vaccine efficacy against aerosol challenge with *F. tularensis*. In Project 2, we will design polyclonal antibody expression libraries against *F. tularensis* and examine the efficacy of passive administration in preventing and treating experimentally induced inhalational tularemia. We will use widely directed polyclonal antibodies to determine overall efficacy, use libraries depleted of putative subversive (blocking) antibodies and generate monospecific polyclonals directed against O-polysaccharides to passively immunize mice and assess protection from aerosol challenge with *F. tularensis*. In Project 3, we will develop three diagnostic systems, using gene amplification and immunochemical detection, to detect *F. tularensis* in clinical and environmental specimens. A transcription mediated amplification (TMA) assay will be developed to detect *F. tularensis* in respiratory secretions and an immunochemical test to detect *Francisella* antigens in respiratory secretions and urine and for monitoring environmental air samples.

Grant: 1U19AI056575-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: JOHNSON, MICHAEL E
Title: Novel Therapeutics for Bacillus anthracis
Institution: UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL
Project Period: 2003/08/15-2008/01/31

DESCRIPTION (provided by applicant): The development of drug-resistant strains of B. anthracis is technically quite feasible, and could be a substantial threat in future terrorist attacks. Building on a well-established collaborative network amongst the investigators participating in this project, we propose an integrated approach toward the development of new antimicrobials, new potentiators of existing antimicrobials, and direct inhibitors of the anthrax toxin as strategies to combat natural and bioengineered forms of B. anthracis. We will use a combination of strategies, beginning with genetic identification and validation of novel bacterial targets, determination of target 3D molecular structures, utilization of diverse chemical libraries for high throughput screening, structure-based drug design, synthesis of lead compounds and their optimization, followed by macrophage and animal testing. An important strength of the application is the broad range of the participants' expertise, including bacterial genetics and biochemistry, structural biology of macromolecules, computer-assisted drug design, synthetic chemistry, macrophage biology, animal modeling and clinical infectious disease. Project 1 will identify and validate new antibiotic targets in ribosomal RNA. Project 2 will identify and validate new infection-related targets in Bacillus anthracis. Project 3 will utilize structure-based design and high throughput screening to develop lead inhibitors of currently known and to-be-identified antibiotic targets. Project 4 will develop inhibitors to prevent the binding of the B. anthracis toxin to the cellular receptor. Project 5 will evaluate the role of antibiotics in modulating cytokine activation and toxin triggering following macrophage infection and animal model development. Four scientific cores will support these projects: A protein expression core will provide proteins for target evaluation and structure-based design. A macromolecular characterization and structure core will provide structural and thermodynamic information for target characterization and structure-based design. A chemical improvement core will provide synthetic design and optimization of lead inhibitors. A bioassay core will provide a variety of assays for identifying and evaluating lead therapeutic agents. An administrative core will provide fiscal management and administrative support.

Grant: 1U19AI056578-01
Program Director: ZOU, LANLING
Principal Investigator: NATARO, JAMES P
Title: Live Vector Vaccines Against Agents of Bioterror
Institution: UNIVERSITY OF MARYLAND BALT PROF SCHOOL BALTIMORE, MD
Project Period: 2003/09/30-2008/01/31

DESCRIPTION (provided by applicant): The United States is under a genuine threat of biological attack. Whereas it has long been known that our enemies were capable of biological assault, only since September 2001 has it become clear that this threat is real. Unfortunately, the US is woefully unprepared to respond to biological attack. While rapid identification of released agents, novel therapeutic interventions and passive immunization will have vital roles to play in biodefense, there is no substitute for pre-existing immunity to the major threats. This immunity can either be provided before such agents are released, or can be provided soon after release has been detected. Unfortunately, for most infectious agents it is not currently feasible to provide rapid administration of vaccines that provide equally rapid protection. Moreover, it will be vital to ensure that any immunization program have both a high level of safety as well as public acceptance. We therefore envision a response strategy which includes live attenuated enteric bacterial vaccines. These vaccines would be very safe, and would elicit both mucosal and systemic responses. After just a single dose, they would protect many exposed to bioattack, and within one week of immunization. The protected would likely be those exposed to small or natural levels of exposure, such as postal workers handling contaminated mail. However, it is anticipated that some victims would be exposed to supra-normal inocula, typically by the aerosol or gastrointestinal routes. For these individuals, a protective vaccination regimen could include priming with the mucosal agent, followed by on-demand boosting with parenterally administered subunit vaccine. After boosting, the recipients would be expected to generate fast, vigorous, balanced immune responses, which would protect them at the level of the mucosa and via both Th1 and Th2 systemic components. This U19 will combine the vast experience of the University of Maryland School Center for Vaccine Development and the Chemical and Biological Defence Center, Porton Down in the development of enteric vaccines against anthrax, plague and botulism. The products developed will be tested in animals and the characteristics of prime-boost responses determined. During the term of the award, we will generate a series of vaccine candidates for immediate Phase 1 human trials.

Grant: 1U19AI056872-01
Program Director: COYNE, PHILIP
Principal Investigator: PETRI, WILLIAM A MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC
Title: Rapid Diagnostics for Category B Enteropathogens
Institution: UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA
CHARLOTTESVILLE
Project Period: 2003/09/30-2008/03/31

DESCRIPTION (provided by applicant): This project is based on a unique decade-long University-Industry partnership in the development and production of stool diagnostic tests for enteric pathogens. Our goal is to produce rapid, sensitive, specific and cost-effective diagnostics appropriate for public health laboratories and point-of-care use for the highest priority Category B enteropathogens. Our hypothesis is that real-time multiplex PCR is the future of enteropathogen diagnostic tests for public health laboratories and that dipstick-formatted antigen detection tests are ideally suited for point-of-care use. Preliminary studies by the Program Investigators have resulted in FDA 510k-approved stool antigen detection tests for Category B agents *Entamoeba*, *Giardia* and *Cryptosporidia*. Project investigators have also pioneered methodology for DNA extraction from stool and therefore have the immediate capability to develop improved and cost-effective diagnostic tests for all 3 of the major biodefense Category B enteric protozoa threat agents. Project 1 will develop stool antigen detection dipstick tests for point-of-care diagnosis of Biodefense Category B Enteric Protozoa and Shiga toxin. Project 2 will develop real-time multiplex PCR using molecular beacons, FRET and/or Taqman probes for public health laboratory diagnosis of Biodefense Category B Enteropathogens. Project 3 will field validate the diagnostic tests produced by Projects 1 and 2 and will correlate protozoal genotype with virulence. The Laboratory Core will provide reagents, testing, and be the clearinghouse for obtaining, culturing and distributing Category B enteric parasites and bacteria for Projects 1-3. The Administrative Core will coordinate activities of the three projects, and facilitate scientific exchange and interactions of investigators. It will also provide administrative oversight and financial accountability. Successful completion of these studies will yield high throughput diagnostic tests that target multiple enteropathogens simultaneously in a single sample.

Grant: 1R01AR048882-01A1
Program Director: MOSHELL, ALAN N.
Principal Investigator: PLANO, LISA R PHD
Title: Molecular analysis of staphylococcal exfoliative toxin A
Institution: UNIVERSITY OF MIAMI-MEDICAL CORAL GABLES, FL
Project Period: 2003/06/01-2008/05/31

DESCRIPTION (provided by applicant): Staphylococcal scalded skin syndrome, SSSS, is a disease primarily of the young and immunocompromised that is rarely seen in healthy adults. SSSS is characterized by specific exfoliation of the upper epidermis in the stratum granulosum of the skin at a site distal to a focus of infection with exfoliative toxin (ET) producing *Staphylococcus aureus*. The exact mechanism of the relative protection of adults or susceptibility of newborns is not known. In an animal model these skin manifestations result from the injection of one of two species-specific exfoliative toxins, ETA or ETB. The mechanism by which these toxins result in exfoliation is now assumed to involve cleavage of desmoglein 1 (Dsg1), a desmosomal protein member of the cadherin family of cell adhesion molecules, by a unique serine protease activity of the exfoliative toxins. Cleavage of this protein fits the clinical picture of SSSS as it is primarily expressed at this layer of this skin. Our hypothesis is that characteristics of the interaction between the exfoliative toxins of *S. aureus* and their target desmoglein 1 explain the species, target and age specificity of these toxins as well as contribute to the pathogenicity of these bacteria. We propose in this study to use molecular techniques to characterize the interaction between the toxin, ETA and the target, Dsg1 of humans and mice. The goal is to determine the domains or amino acids of Dsg1 needed for recognition and cleavage by ETA and the domains or amino acids of ETA responsible for target binding and localization to the skin. Results from these analyses will provide insight into the mechanism for the age and species specificity of this unique serine protease. We further propose to develop an animal model of staphylococcal impetigo to address the role of the exfoliative toxins in pathogenesis of these bacteria.

Grant: 1R03AR048265-01A1
Program Director: GRETZ, ELIZABETH
Principal Investigator: ANGUITA, JUAN PHD
Title: p38 MAP kinase role in the genesis of Lyme arthritis
Institution: UNIVERSITY OF NORTH CAROLINA CHARLOTTE, NC
CHARLOTTE
Project Period: 2003/06/01-2006/05/31

DESCRIPTION (provided by applicant): *Borrelia burgdorferi* is the causative agent of Lyme disease, the most common vector-borne disease in the United States. Among the symptoms occurring as a result of infection with *B. burgdorferi*, Lyme arthritis is the most prevalent in patients infected with the spirochete. *B. burgdorferi* infection causes an upregulation of proinflammatory cytokines. Tumor necrosis factor (TNF) alpha, IL-12 and Interferon (IFN) gamma, followed by a CD4+ T cell helper (Th) type 1 cytokine production pattern have been reported in strains susceptible to develop more severe inflammatory symptoms. The molecular mechanisms by which the proinflammatory cytokine pattern is induced are not completely elucidated. p38 MAP kinase is involved in the genesis of several non-infectious arthritides, like rheumatoid arthritis, by regulating the expression of proinflammatory cytokines, p38 MAP kinase is also required for the production of IFNgamma, an important proinflammatory mediator, by T cells. The use of a specific inhibitor is considered a potential therapy in those processes that involve a proinflammatory response mediated by p38 MAP kinase. We hypothesize that *B. burgdorferi* activates the p38 MAP kinase pathway. The activation of this pathway is required for proinflammatory cytokine production by macrophages and neutrophils. The activation of this pathway is also required for IFNgamma production by CD4+ T cells and Th1 differentiation during Lyme borreliosis. Thus, we propose that the p38 MAP kinase pathway is required for the development of murine Lyme arthritis. Understanding the contribution of this pathway to the development of murine Lyme arthritis, as a model of human disease, may open new therapeutic approaches that would take advantage of specific pharmacological inhibitors of p38 MAP kinase currently being tested in human clinical trials.

Grant: 1R03AR049383-01
Program Director: GRETZ, ELIZABETH
Principal Investigator: PARVEEN, NIKHAT PHD
Title: DbpA/B proteins of *Borrelia burgdorferi* & Lyme arthritis
Institution: UNIV OF MASSACHUSETTS MED SCH WORCESTER, MA
WORCESTER
Project Period: 2003/03/01-2006/02/28

DESCRIPTION (provided by applicant): Lyme disease presents a unique clinical system to study cellular and molecular mechanisms responsible for chronic inflammatory diseases. The disease, caused by the spirochete *Borrelia burgdorferi*, is the most prevalent arthropod borne disease in the United States. It is a multisystemic illness that affects skin, muscles, joints, heart and nervous system. If left untreated, chronic manifestations are frequently observed and Lyme arthritis is the most common symptom in North America. My long term goal is to identify the virulence factors of *B. burgdorferi* involved in attachment to host cells and in colonization of various tissues, and characterize their role in the pathogenesis, diagnosis and prevention of chronic Lyme disease. Glycosaminoglycans (GAGs), ubiquitously expressed on the surface of all nucleated cells, are recognized by various Lyme spirochetes and several bacterial molecules are involved in this adherence. Decorin binding lipoproteins DbpA and DbpB of *B. burgdorferi* show affinity for heparin and dermatan sulfate GAGs in addition to the proteoglycan decorin. My hypothesis is that DbpA and DbpB contribute to the colonization of various tissues by *B. burgdorferi* binding to GAGs and decorin present on the host cells and trigger an inflammatory response in skin and joints causing erythema migrans and Lyme arthritis. The major question to be addressed in this study are: (1) Do DbpA and DbpB contribute to the GAGs-mediated attachment of *B. burgdorferi* to host cells and to the inflammatory response in the joints of susceptible mice? (2) Does deletion of *dbpA* and *dbpB* genes affect attachment of *B. burgdorferi* to the host cells? (3) Are DbpA and DbpB lipoproteins essential virulence factors of *B. burgdorferi* that trigger Lyme arthritis? Significance: Lyme arthritis exhibits several symptoms similar to those of rheumatoid arthritis. However, unlike rheumatoid arthritis, the causative agent is known in Lyme disease and hence, it is feasible to analyze the molecular mechanisms involved in this form of destructive arthritis. In addition, *B. burgdorferi*/infected mouse exhibits symptoms similar to those of human Lyme disease, and hence, murine model provides an ideal system to analyze the mechanisms of Lyme borreliosis. This study will characterize the role of two spirochete lipoprotein adhesins in Lyme arthritis in the murine model.

Grant: 1R21AR049263-01A1
Program Director: PANAGIS, JAMES S.
Principal Investigator: HUDSON, MICHAEL C
Title: S. aureus Enhances Interaction with Biomaterials
Institution: UNIVERSITY OF NORTH CAROLINA CHARLOTTE, NC
CHARLOTTE
Project Period: 2003/09/25-2005/06/30

DESCRIPTION (provided by applicant): The hypothesis to be tested in the current proposal is that killed *S. aureus* enhances the interaction of osteoblasts with biomaterials. The specific aims are the following: 1. Does UV-killed *S. aureus* enhance attachment of osteoblasts to thin films of titanium alloy? Polystyrene tissue culture dishes will be coated with titanium alloy Ti-6Al-4V (Ti) using thermal vapor evaporation. The Ti-coated culture dishes will be coated with fibronectin (Fn), and incubated with UV-killed *S. aureus* strain UAMS-4. Culture dishes will then be rinsed to remove unattached bacteria, followed by culture of normal human osteoblasts in the coated dishes. Attachment of osteoblasts to Ti in the presence and absence of UV-killed *S. aureus* will be analyzed using phosphor-screen autoradiography. The strength of osteoblast attachment will be analyzed using micropipette aspiration techniques. Results will be compared to cultures of osteoblasts grown in dishes coated with Ti and Fn. 2. Does UV-killed *S. aureus* enhance spreading and proliferation of osteoblasts, and increase type I collagen synthesis on thin films of Ti alloy? Normal human osteoblasts will be cultured in Ti/Fn/*S. aureus*-coated dishes. Osteocalcin expression will be examined to assess spreading of the osteoblasts on the biomaterial. Amounts of alkaline phosphatase activity and ³H-thymidine incorporation will be assessed and used as indicators of osteoblast viability and proliferation, respectively. Type I collagen synthesis will be measured to assess production of bone matrix. Results will be compared to osteoblasts cultured in Ti/Fn-coated dishes. 3. Does UV-killed *S. aureus* enhance interfacial shear strength between bone and titanium implants in vivo? An in vivo model is proposed to address this aim. Titanium wire coated with Fn and UV-killed *S. aureus* will be inserted into the femoral canal of rats. Ti wire coated with Fn will be used as controls. Femurs will be harvested at different weeks following insertion. The implant interface will be examined using transmission electron microscopy and the interfacial shear strength assessed using a pull-out test.

Grant: 2P01CA028842-18A2
Program Director: PATEL, APPASAHEB R.
Principal Investigator: CORREA, PELAYO MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC
Title: Etiological Studies of Gastric Carcinoma
Institution: LOUISIANA STATE UNIV HSC NEW NEW ORLEANS, LA
ORLEANS
Project Period: 1981/06/01-2008/06/30

DESCRIPTION (provided by applicant): The goal of this Program Project has been and continues to be the multi-disciplinary study of the etiology of gastric cancer. This neoplastic disease is second only to lung cancer in incidence and mortality worldwide. In the United States gastric cancer rates have decreased considerably. There are, however, high-risk groups, especially African Americans, Amerindians, and immigrants from Asia, Northern/Eastern Europe and Latin America. It has become increasingly clear that a major etiologic factor is chronic infection with *Helicobacter pylori*. About one half of today's world population is infected, especially groups of lower socioeconomic status. The International Agency for research on Cancer has classified *Helicobacter* infection as a class 1 carcinogen. There are great differences in the outcome of the infection. Most infections are mild and subclinical. Clinical infections may lead to duodenal ulcer accompanied by non-atrophic gastritis, which does not increase gastric cancer risk, or to multifocal atrophic gastritis, which may lead to gastric ulcer and gastric cancer. Our general hypothesis is that the immune and inflammatory responses determine the outcome of the infection. Our Program Project explores the dynamics of the response with immunologic and histopathologic techniques in adults and children (Project 1,2 and 3). Two epidemiologic projects are also proposed: 1) follow-up of the chemoprevention cohort, which explores the natural history of infection after eradication attempts (Project 1); and 2) study of the dynamics of infection and reinfection in children of a hyper-endemic area in search for answers to the critical events in initiating the possible carcinogenesis pathway, namely persistence of infection in childhood. (Project 3). COLLABORATING INSTITUTION(S): Delft laboratories, The Netherlands Emory University Medical Center Atlanta, GA University del Valle, Cali, Colombia University de Narino, Colombia University de Antioquia, Medellin, Colombia University of Texas School of Public Health, Houston TX APR NOTE: This Program Project Grant has addressed the etiology of gastric cancer for 20 years and is in the fifth cycle of funding. This competitive renewal application continues the unique and multidisciplinary study of gastric cancer. The general hypothesis put forward by this Program Project is that the immune and inflammatory responses determine the outcome of the *Helicobacter pylori* infection leading to gastric ulcer or gastric cancer. The Program includes 3 Projects and 4 Cores. It was felt at the accelerated review that the investigators had resolved all the problems identified in the last review. The Program Project has continued to build on the broad clinical, pathological, and molecular experience accumulated by the Principal Investigator and his program project staff. Two unique populations of *H. pylori*-

infected individuals located in Colombia are being studied. In one population non-atrophic gastritis (NAG) is more common along with low gastric cancer rates and in the second population multi-focal atrophic gastritis (MAG) is more common with a much higher gastric cancer rate. A major strength of this research and the Program Project is the investigators' matchless understanding of the etiology of gastric cancer in these unique and well-characterized populations. These two populations (a major world-wide resource for studying *H. pylori* pathogenesis) constitute the major strength of this application along with the more than 18 years of study of this gastric cancer problem by the Principal Investigator in a program project environment. In a previous review there were problems with some of the work not being adequately described for an accurate assessment of its feasibility, but these deficiencies have been eliminated. The program is totally unique, has been highly successful in the past, and should make substantial progress in this new funding period. The recommended merit scores of all three projects was 1.4. Three of the cores, Histopathology, Administrative and Field Activities, and Genetic Characterization are rated superior, and the Administrative and Data Management Core is rated satisfactory. The Program is highly integrated and in a very special way makes the whole more valuable than the parts. This was a unanimous observation by the reviewers. The Program is recommended for 5 years of funding. Project 1, "Chemoprevention of gastric Dysplasia", is led by Elizabeth Fontham, Ph.D. The focus of this project continues to be an important population from Colombia who are at high risk for gastric cancer and who have been the focus of this program project since its inception. A unique cohort of subjects with MAG from this population were the subjects of an interventional study in the 1990's to examine the effect of beta carotene and Vitamin C and/or eradication of *H. pylori* on the progression of gastric precancerous histological lesions. Contact has been maintained with these subjects, around half of who are now *H. pylori*-negative. This project will continue to follow these subjects closely by endoscopy and clinical evaluation to determine whether the continuing natural history of progression in gastric preneoplasia is altered by the persistent eradication of *H. pylori*. A secondary aim will be to determine whether those subjects who become reinfected by *H. pylori* are infected by less virulent strains, as suggested by preliminary data. The project has many strengths, including its focus on a unique and well-defined clinically relevant population, and the expertise of the clinicians and pathologists, who have proven their ability to work cohesively under the supervision of Drs Correa and Fontham over many years. This project received an average merit rating of 1.4. Project 2, "Immune Response to *H. pylori* in Non-atrophic Gastritis and Multifocal Gastritis" is led by Augusto Ochoa, M.D. It has continued to improve since the first review. One major exception was the validation of using PBL responses to reflect the immune and inflammatory status of leukocytes in the gastric mucosa. This was addressed satisfactorily in the accelerated peer review. The investigators responded by stating that for the first third of the patients analyzed (numbering 20), in vitro PBL responses will be compared with in situ gastric tissue responses. If concordance is observed, the remaining patients in the study will be followed as initially proposed, with concentration of efforts on PBL analysis. If, on the other hand, concordance between the PBL vs. in situ tissue analysis is not observed, the investigators will be able to adjust their analysis to include both PBL's and in situ analysis of all remaining subjects. This response is entirely appropriate and alleviates the biggest uncertainty in the approach taken in Project 2 during the previous submission. The greatest strengths of this project include the unique patient resources available and the previous productivity of the investigators. The overall goal of defining differences in the host immune response between *H. pylori*-infected patients at risk of developing gastric cancer versus duodenal ulcers is very worthwhile, and within the capability of the investigators. The project has the potential to help dissect the relative contributions of host and bacteria to the development of gastric cancer. This project received an average merit rating of 1.4. Project 3, "Community Intervention-Follow-up of Colombian Children" is led by Karen Goodman in a consortium arrangement with The University of Texas School of Public Health. This project addresses important questions in an appropriate fashion. In

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

the previous version of this project, a clerical effort resulting in the reviewers not seeing the final draft. This problem has been resolved with many of the perceived scientific problems also being clarified. The 3-drug therapy chosen was identified by the reviewers as "a peculiar combination". In the most recent submission, Metronidazole has been added to create a 4-drug therapy. This regimen is consistent with contemporary medical practices. This project received a merit rating of 1.4 Core A, Histopathology, is led by the Principal Investigator, Dr. Pelayo Correa. This laboratory will perform all the histological and histochemical processing and evaluation of the numerous biopsies taken from each of the projects. It is a critically important core for this program project. It will be essential for all three projects, especially Project 1. This laboratory has proven over many years that it is ideally equipped for these purposes, and Dr Correa, the Core director, has an unequalled expertise in the interpretation of gastric pathology. This is a superior core. Core B, "Genetic Characterization of *H. pylori* Strains" led by Barbara Schneider, Ph.D., provides resources for genotypic characterization of three putative virulence genes in *H. pylori* strains. . The LiPA assay for this purpose is well validated and supported by the experience of its inventor, Dr. van Doorn, who will serve as a consultant. The high-throughput advantages of the LiPA assay will be exploited in Projects 1 and 2. It is not clear whether *babA* typing will also be done by LiPA or by other, independent PCR's. In addition development of non-invasive genotyping methods using fecal samples is proposed. This core received a superior rating. Core C, "Administrative and Data Management (New Orleans)" is led by Dr. Pelayo Correa, M.D. who is also the Principal Investigator of this grant application. This administrative effort has been quite successful in the past and is very well organized and efficient. This Core had the deficiency during the last review of an underpowered statistical analysis effort. Dr. Correa has addressed this deficiency, and both Ms. Du and Ms. Camargo have been assigned to work under Dr. Mera. The question during the last review was regarding the amount of time Dr. Mera could devote to this Program Project. His credentials are perfectly matched for this Program Project, but his time available was considered inadequate because of the large amount of statistical analysis needed by this Program Project. To satisfy this criticism, two new faculty were recruited the biostatistics area. One of these individuals, Dr. Velasco, is Spanish speaking and could help the program project and reports to Dr. Fontham, the Project Leader of Project 1. Most importantly, the new head of the Cancer Center Statistical Department (just hired the week of the current review at the full professor level) will give 15 percent of his time to this Program. This individual is highly qualified and very experienced with the types of statistical problems that will occur in these studies. Thus, the program project statistical effort is going to be run 25 percent time by Dr. Mera and 15% by the new senior faculty member with 2 capable support people at LSU. This is a strong addition to the Core and resolves the major statistical problem from the last review. This core received a satisfactory rating. Core D, "Administrative and Field Activities (Colombia)" is led by Luis Eduardo Bravo, M.D. As was stated previously this is an outstanding core. The cost effectiveness of this effort is remarkable. Past history of this effort and the intact staff from the previous funding period make this core effort convincing and very workable. Some of the details missing about data flow and quality assurance from the previous review were not entirely provided in this new submission, but the effort is still superior, as the overall coordination between the various units in Colombia and between Colombia and the US look strong. This core received a superior rating. Commentary related to Progress in the current funding period, Integrated Effort, Principal Investigator, Support to be negotiated for replacement and Human Subjects are unchanged from the previous review. REVISION NOTE: Modified to include review panel roster. INDIVIDUAL PROJECTS AND CORES PROJECT 1:

Chemoprevention of Gastric Dysplasia: Long-term follow-up of a cohort treatment for *H. pylori* infection (Elizabeth T.H. Fontham, Dr. Ph.H., 15 percent effort)

Grant: 2R01CA065875-09
Program Director: DUBOIS, RONALD J.
Principal Investigator: COLEMAN, ROBERT S PHD
Title: Azinomycins - Total Synthesis and Mechanism of Action
Institution: OHIO STATE UNIVERSITY COLUMBUS, OH
Project Period: 1995/05/01-2007/11/30

DESCRIPTION (provided by applicant): Azinomycins A and B are potent and effective antitumor agents isolated from Streptomyces. Their biological activity resides in their ability to covalently alkylate and subsequently cross-link double stranded DNA. There has been no developmental work on the natural agents because of their poor availability from natural sources and because of their chemical instability. The aim of this proposal is to elucidate the molecular details involved in the expression of cytotoxicity and antitumor activity by these agents through total synthesis of the natural products and a rationally designed series of structurally and functionally related agents. The unprecedented structure, intricate functionalization, unique molecular mechanism of action, and effective antitumor activity make the azinomycins attractive targets for study. The major rationale for synthetic efforts lies in the construction of structurally and functionally related agents for use in elucidation of the details of covalent interaction of these agents with DNA. Methodology developed in the course of synthetic efforts will be used to design and synthesize agents with which to explore the chemical events surrounding DNA cross-linking. A convergent synthetic approach brings together five small fragments in a series of ester, amide, and olefin bond formation events and will be the key to the modular construction of a variety of molecules with which to explore structure-function relationships based on the azinomycin skeleton.

Grant: 2R01CA077955-06
Program Director: DASCHNER, PHILLIP J
Principal Investigator: PEEK, RICHARD M MD
Title: H.Pylori Relationship to Digestive Diseases and Cancer
Institution: VANDERBILT UNIVERSITY NASHVILLE, TN
Project Period: 1997/09/30-2008/03/31

DESCRIPTION (provided by applicant): *H. pylori* colonization increases the risk for gastric adenocarcinoma yet only a fraction of infected persons ever develop cancer. *H. pylori* strains that contain the *cag* pathogenicity island (*cag*+) augment the risk for severe gastritis and gastric cancer, and we have shown that *cag* genes are required for the development of inflammation in a rodent model of *H. pylori*-induced gastric cancer. Our data also demonstrate that *cag* genes are necessary for induction of proinflammatory cytokine release and apoptosis in gastric epithelial cells in vitro, events mediated by NF-(B and/or mitogen-activated protein kinases (MAPK). In vivo, however, heterogeneity exists among apoptosis scores within infected populations, and one explanation for such variability is *H. pylori* genetic diversity. Gastric epithelial cell proliferation is higher but apoptotic indices are lower among persons colonized with *cag*+ compared to *cag*- strains or uninfected persons, and reduced rates of cell loss, when accompanied by hyperproliferation, may heighten retention of mutagenized cells, which could predispose towards malignancy. One host effector that may influence carcinogenic pathways associated with *H. pylori* is matrilysin, a matrix metalloproteinase that enhances tumor formation, and is over-expressed in premalignant and malignant lesions within *H. pylori*-infected human mucosa. Constitutive expression of matrilysin selects for cells with a reduced sensitivity to apoptosis, and we now show that matrilysin is detected exclusively in human mucosa colonized by *cag*+ strains, and these strains selectively induce matrilysin in vitro through *cag*-mediated activation of NF-(B and MAPK. Another host pathway through which inflammatory mediators may influence pathogenesis is PPAR(, a nuclear transcription factor. Our data now show that PPAR(activation inhibits *H. pylori*-induced NF-(B signaling in vitro, and attenuates inflammation and injury in rodent models of *H. pylori*-induced gastritis. Our hypothesis is that strain-selective activation of NF-(B and/or MAPK by *cag*+ strains regulates matrilysin expression which may contribute to the augmentation in carcinogenic risk associated with these strains by attenuating apoptosis within colonized mucosa, and that PPAR(activation suppresses phenotypes related to carcinogenesis by inhibiting *H. pylori*-induced NF-(B-mediated responses in gastric epithelial cells. To address this, our specific aims are: 1) To determine the effects of *H. pylori* and mutant strains on matrilysin-dependent cellular responses related to carcinogenesis in vitro and in matrilysin deficient mice; 2) To identify targets of PPAR(that suppress *H. pylori*-induced epithelial responses associated with oncogenesis; and 3) To determine the role of PPAR(in regulating inflammatory and injury responses to *H. pylori* using in vivo models of PPAR(pharmacologic activation and genetic deficiency. Studies of these variables in vitro and in animal systems that reflect pathogenesis in humans will not only improve our understanding of *H. pylori*-induced carcinogenesis and facilitate identification of potential therapeutic targets, but may also provide insights into other malignancies that arise within the context of inflammatory states.

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Grant: 1R01CA090841-01A2
Program Director: ARYA, SURESH
Principal Investigator: SAWICKI, JANET A
Title: Targeted Death of Prostatic Cancer Cells
Institution: LANKENAU INSTITUTE FOR MEDICAL RESEARCH WYNNEWOOD, PA
Project Period: 2003/04/01-2005/11/30

DESCRIPTION (provided by applicant): The standard therapy for men with metastatic prostate cancer is to reduce tumor size by androgen ablation, either by bilateral orchiectomy or the use of luteinizing hormone-releasing hormone analogues. While many prostate cancer cells die in the absence of androgens, some cells are androgen-independent and do not require androgens for survival. With time, the surviving cells begin to grow aggressively. The result is that most men receiving this therapy develop recurrent tumors and die within two years. To improve the effectiveness of this therapy, we hypothesize that following androgen ablation therapy for the treatment of prostatic carcinoma, application of a regulated recombination system to target expression of diphtheria toxin (DT-A) to androgen independent cancer cells would be an effective way to arrest the development of recurrent tumors. We propose a strategy to use replicative-defective adenoviral vectors to deliver DT-A specifically to androgen-independent prostate cancer cells. The regulated expression of this highly toxic protein in cells will result in their death. We shall test the effectiveness of this approach in cultured human prostate cancer cells, in xenografts in mice developed from such cells, and in prostate tumors in a transgenic mouse model. We expect our investigations will lead to the development of a novel gene therapy for prostate cancer patients that will effectively arrest the development of recurrent tumors.

Grant: 2R01CA094426-06A1
Program Director: HALLOCK, YALI
Principal Investigator: SHEN, BEN PHD
Title: Biosynthesis of hybrid peptide-polyketide antibiotics
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 1996/12/01-2007/12/31

DESCRIPTION (provided by applicant): Nonribosomal peptide synthetases (NRPSs) and polyketide synthases (PKSs) have been exploited successfully in combinatorial biosynthesis of "unnatural" natural products for drug lead discovery and optimization. Hybrid NRPS-PKS systems will allow the production of novel metabolites by incorporating both amino acids and carboxylic acids, greatly expanding the size and structural diversity of resulting combinatorial biosynthetic libraries. A great challenge in constructing hybrid NRPS-PKS systems lies in the revelation of the molecular basis for intermodular communication between NRPS and PKS. I propose to continue a study of the biochemistry and genetics of the production of the bleomycins (BLMs) and related metabolites as a model for hybrid peptide-polyketide natural product biosynthesis. My long-term goal is to construct hybrid NRPS-PKS systems to produce novel cell-permeable bioactive molecules and pharmacological leads. My short-term goals for this project are (1) to biochemically and genetically characterize the BLM biosynthetic pathway and (2) to make novel BLM congeners through combinatorial biosynthesis for the discovery and development of clinically useful anticancer drugs. My hypotheses are: (1) the BLM megasynthetase is a "natural" hybrid NRPS-PKS system, the studies of which will reveal how nature integrates NRPS and PKS proteins into a hybrid NRPS-PKS system; (2) the initiation and termination of BLM biosynthesis and the formation of the bithiazole moiety of BLM are unprecedented in peptide biosynthesis, the characterization of which will uncover novel chemistry for NRPSs; (3) the BLM megasynthetase provides a novel platform for combinatorial biosynthesis to make clinically valuable anticancer drugs. My specific aims are: (1) functional analysis of the BLM biosynthetic gene cluster in vivo; (2) biochemical characterization of the BLM megasynthetase in vitro; (3) production of novel BLMs by engineering BLM biosynthesis and evaluation of them as anticancer drugs. The outcomes of these studies will (1) expand the repertoire of NRPS and PKS genes for combinatorial biosynthesis, (2) lay the foundation for rational construction of hybrid NRPS-PKS systems, and (3) potentially lead to the production of novel BLMs with improved therapeutic efficacy as anticancer drugs.

Grant: 1R01CA095137-01A1
Program Director: HOWCROFT, KEVIN
Principal Investigator: LANIER, LEWIS PHD IMMUNOLOGY, OTHER
Title: RAE-1 Family of Proteins in Innate and Adaptive Immunity
Institution: UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA
Project Period: 2003/02/14-2008/01/31

DESCRIPTION (provided by applicant): Natural killer (NK) cells participate in the innate immune response against pathogens and tumors. There is growing evidence that they are involved in allograft rejection and possibly in certain autoimmune diseases. The receptors on NK cells responsible for their activation are only now being appreciated. One of these receptors, NKG2D, has been implicated in the ability of NK cells to kill certain tumors and virus-infected cells. NKG2D is present on all NK cells, CD8+T cells, gamma delta-TcR + T cells and some activated macrophages. Recently, we and others cloned a family of mouse genes, designated RAE-1, encoding MHC class I-like proteins that bind with high affinity to mouse NKG2D. Human orthologs of the RAE-1 genes (designated as either ULBP or RAE-1-like genes) have been identified and the proteins encoded by these genes are recognized by the human NKG2D receptor. In humans, the NKG2D receptor also recognizes MICA and MICB, polymorphic MHC class I antigens that are induced by stress, transformation and viral infection. Emerging evidence suggests that the RAE-1 and MIC glycoproteins may serve to trigger the innate immune responses mediated by NK cells and gamma delta-TcR +T cells and function to co-stimulate antigen-specific responses by CD8 + cytotoxic T lymphocytes. We propose to investigate the RAE-1 family of genes and determine their role in innate and adaptive immune responses. The specific aims of this program are: 1) to determine whether the RAE-1 genes are polymorphic, analyze their expression and determine if they elicit allogeneic responses, 2) to establish the relevance of induction of the RAE-1 molecules during viral infection, to determine how the human CMV UL 16 protein and potentially other viral proteins interfere with RAE-1, and to evaluate whether viruses other than HCMV induce the RAE-1 genes, and 3) to evaluate the mechanisms responsible for the induction of the RAE-1 genes in normal cells. The overall objective of this project is to understand both the beneficial and potentially adverse functions of the RAE-1 family of antigens in innate and adaptive immune responses.

Grant: 1R01CA098309-01
Program Director: STARKS, VAURICE
Principal Investigator: KATO, IKUKO MD
Title: Genetic Susceptibility to Infection Related Cancer
Institution: WAYNE STATE UNIVERSITY DETROIT, MI
Project Period: 2003/02/01-2006/01/31

DESCRIPTION (provided by applicant): The long-term goal of the proposed study is to provide a scientific basis to develop efficient primary prevention strategies against infection/inflammation-related cancers. Mounting evidence suggests that a variety of infectious agents have a role in the pathogenesis of human cancers. It is estimated that 15.6 percent of the worldwide cancer incidences in 1990 can be attributed to these infectious agents, accounting for a total of 1,450,000 cases. *Helicobacter pylori* (HP) is ranked top among various infectious agents and represents approximately 5 percent of new cancer cases in the world. These cancers are important from a public health point of view because they are potentially preventable by antibiotics treatment or vaccination. Whereas HP infection is very common (80-90 percent) in populations with high risk for stomach cancer, it is known that only a very small fraction of the population infected with HP actually develops cancer, suggesting a role for genetic components in HP-related carcinogenesis in addition to that of environmental co-factors. This proposal will specifically focus on the 2 groups of polymorphic genes, receptors to HP lipopolysaccharide (LPS), a cell wall component, which elicits immediate proinflammatory responses, (1) CD14 (C-260T), (2) TLR4 (A896G) and (3) NOD2 (3020insC); and resultant cytokines, (4) IL-8 (T-251A), (5) MCP1 (G-2518A), (6) IL-1beta (T-31C) and (7) TNF-alpha (G-308A). These polymorphic genes are known to be functional and have been postulated to modify host responses to HP infection. The proposed study will be designed as a spin-off study of a chemo prevention trial for gastric cancer in Venezuela, taking advantage of unique characteristics of the study population, i.e., a strikingly high (95 percent) HP infection rate and high prevalence of gastric premalignant lesions. It will utilize biological specimens and epidemiological and histopathological data collected at the baseline examination from the 2200 participants. Genomic DNA will be isolated from these specimens and tested for the polymorphic genes listed above. The specific aim of the proposed study is to evaluate whether those genotypes or alleles of the polymorphic genes which lead to greater responses to HP infection are associated with increased risk of high-grade gastric precancerous lesions. Secondary aims include to examine histopathological correlates of these polymorphisms and to determine whether selected environmental factors modify the above associations.

Grant: 1R01CA101931-01
Program Director: DASCHNER, PHILLIP J
Principal Investigator: MCGEE, DAVID J
Title: H.pylori arginase modulation of ulcers and cancer
Institution: UNIVERSITY OF SOUTH ALABAMA MOBILE, AL
Project Period: 2003/07/18-2008/06/30

DESCRIPTION (provided by applicant): *Helicobacter pylori* infects approximately 50% of the human population, causing severe gastric diseases including gastritis, peptic ulcers, and gastric cancer. *H. pylori* exerts an enormous amount of cellular energy on nitrogen metabolism including the urease enzyme. Therefore, the critical role of urease in virulence has been a major research focus. However, *H. pylori* has a substantial number of other nitrogen metabolizing proteins, whose role in virulence and maintaining nitrogen balance are poorly understood. Here, the focus is arginase, an enzyme metabolically upstream of urease that converts arginine to urea and ornithine. Polyamines (produced from ornithine) and arginase are elevated in the gastric mucosa of gastric cancer patients. Preliminary data demonstrate arginase is critical for *H. pylori* survival from acid and nitric oxide (NO), two innate host defenses. Furthermore, gerbils infected with wild type *H. pylori* develop gastritis and ulcers, whereas no pathologies are observed in gerbils infected with the isogenic arginase mutant. The central hypothesis of this proposal is that *H. pylori* arginase inhibits host NO, elevates polyamines, and contributes to gastritis, ulcers and cancer. To test this hypothesis, there are two specific aims: 1) Determine roles of *H. pylori* arginase in virulence in tissue culture models and 2) Determine the roles of *H. pylori* arginase in virulence using gerbils. In aim 1, levels of arginase needed to inhibit macrophage NO and elevate polyamines will be assessed. Arginase-dependent cytokine profiles will be identified. In aim 2 the role of arginase in virulence in the gerbil model will be assessed by determining whether arginase contributes to gastric cancer or ulcer development, induces an immune response, affects host NO, arginase or polyamine levels, and protects gerbils from *H. pylori* challenge. The proposed experiments will significantly enhance our understanding of the roles of *H. pylori* arginase in protection from innate defenses as well as roles in ulcer and cancer development and optimization of nitrogen levels in vivo.

Grant: 1R03CA099512-01A1
Program Director: STARKS, VAURICE
Principal Investigator: PEREZ-PEREZ, GUILLERMO I MD
Title: Immune response to Helicobacter pylori VacA as a marker
Institution: NEW YORK UNIVERSITY SCHOOL OF MEDICINE NEW YORK, NY
Project Period: 2003/08/06-2005/03/31

DESCRIPTION (provided by applicant): Although gastric cancer mortality rates are gradually decreasing around the world, there are countries, especially in East Asia in which this disease remains prevalent. Helicobacter pylori colonization has been strongly associated with gastric cancer in cohort and case-control studies. Until now, most studies have reported an association between H. pylori and gastric cancer using serological surveys with pools of cell surface preparations from the bacteria as the antigen. However, H. pylori isolates from different geographic areas vary in genotype. We propose to design specific antigenic regions from the major virulence marker of H. pylori (VacA), and by assessing the immune response to this bacterial antigen, determine whether it is a better predictor for the risk of development of gastric cancer in selected populations. For VacA we will obtain peptide antigens specific for the mid region (m1 and m2) because preliminary data suggests that differences in this region may be a predictor of the development of gastric cancer. From a nested case-control study of Japanese-Americans in Hawaii, serum samples were obtained from individuals before they developed gastric cancer over a 21-year follow-up. We also have serum samples from control individuals who did not develop gastric cancer. We expect to find that the immune response to one particular vacA genotype (m1) is predominantly observed in those patients who developed gastric cancer compared to those in whom gastric cancer did not develop. If our hypothesis is correct, we hope to establish a simple and economical serological test to assess the risk of gastric cancer development.

Grant: 1R03CA103095-01
Program Director: DASCHNER, PHILLIP J
Principal Investigator: MAIER, ROBERT J BS
Title: Virulence Determinants in *Helicobacter hepaticus*
Institution: UNIVERSITY OF GEORGIA ATHENS, GA
Project Period: 2003/07/01-2005/06/30

DESCRIPTION (provided by applicant): The presence of *H. hepaticus* in the liver of mice is correlated with the development of hepatitis and liver carcinomas, and these symptoms can be studied by inoculating mice with the bacterium. The specific aim of the proposed work is to begin to identify some of the bacterial factors that may be virulence components in *H. hepaticus* via a targeted mutagenesis approach. A complete genome sequence for the type strain is expected to be available sometime in 2003, and some suspected virulence factors (superoxide dismutase, alkyl hydroperoxide reductase, and hydrogenase) have been identified by enzyme assays on *H. hepaticus* extracts (in the Principal Investigator's lab). The above three enzymes have been shown to affect virulence in the related bacterium *Helicobacter pylori* (*H. pylori*), by the same approach (targeted mutagenesis) proposed here. The sequences for the three genes (*sodB*, *ahpC*, and *hydA*) are being made available to the Principal Investigator prior to the full genome sequence release so that targeted mutants can be generated in these three potential virulence factors. However, a mutant *H. hepaticus* has never been recovered in any gene. Therefore, an initial pilot study is needed to ascertain the feasibility of this approach, using kanamycin-resistance cassette *alMic* exchange mutagenesis (the method that is currently used for *H. pylori*). Once mutants are made they will be compared to the parent strain for colonization and infections of mice, and the resulting liver tissue will be assessed for disease by a board certified veterinary pathologist. If specific mutants can be created, then virulence determinants can begin to be systematically studied.

Grant: 1R03CA103492-01
Program Director: ERICKSON, BURDETTE (BUD) W
Principal Investigator: PARSONNET, JULIE
Title: Microarrays of Intermediate Endpoints of Gastric Cancer
Institution: STANFORD UNIVERSITY STANFORD, CA
Project Period: 2003/09/20-2005/08/31

DESCRIPTION (provided by applicant): *Helicobacter pylori* (*H. pylori*) infection causes gastric adenocarcinoma, the second leading cause of cancer mortality worldwide. Screening and treatment of *H. pylori* could be a cost-effective way of preventing cancer, but whether *H. pylori* eradication can stop progression of gastric carcinogenesis remains unknown. Several studies have been completed that indicate incomplete regression of gastric preneoplastic conditions with *H. pylori* eradication. Using preneoplastic conditions as surrogate markers, however, is problematic in that regressions of surrogates may not signify regressions in cancer risk. To know the meaning of microscopic regression, one must look at the molecular underpinnings of the observed phenotypic changes. We hypothesize that the histologic regression observed with *H. pylori* eradication reflects molecular changes. To determine if this is the case we will dissect the role that *H. pylori* plays in molecular pathways of gastric preneoplasia. cDNA microarray will be used to assess gene expression profiles of archived tissues from a randomized placebo-control trial of *H. pylori* eradication conducted in Mexico. A total of 90 subjects (45 from the treatment arm and 45 from the placebo arm) with preneoplastic conditions (gastritis, atrophy, and metaplasia) will be selected from the original trial. For each of the 90 subjects, a pair of pre- and post-treatment gastric tissues will be used for cDNA microarray. The resulting gene expression data will be analyzed by hierarchical clustering and Significant Analysis of Microarrays (SAM). In addition to the data on patients with preneoplastic lesions, we also have access to gene expression profiles of 90 gastric cancer tissues and 22 normal gastric tissues from investigators at our institution. Our data can then bridge the gap between different carcinogenetic stages between normal and cancer. In summary, the primary aims of this study are: 1) to assess the changes of global gene expression patterns associated with *H. pylori* eradication and identify reversible, molecular "intermediate biomarkers" for gastric cancer, 2) to compare the results from gene expression studies to those from pathologic diagnoses, and 3) to compare the results from gene expression studies in normal, preneoplastic, and malignant tissues. This proposed study will not only help elucidate the poorly understood molecular mechanisms of gastric carcinogenesis, but also help delineate the role of *H. pylori* infection in this process. Despite National Institutes of Health (NIH) guidelines to the contrary, *H. pylori* screening and eradication therapy is being widely used by primary care practitioners in the hope that it will prevent cancer in high-risk populations. We hope our results will provide impetus for more rational screening and treatment policies, particularly for the minority populations in the United States and people in developing countries who suffer most from this highly fatal disease.

Grant: 1R15CA100088-01
Program Director: HALLOCK, YALI
Principal Investigator: CRUPPER, SCOTT S PHD
Title: Overproduction of Epothilones in *Sorangium cellulosum*
Institution: EMPORIA STATE UNIVERSITY EMPORIA, KS
Project Period: 2003/09/16-2006/08/31

DESCRIPTION (provided by applicant): Epothilones are a microtubule stabilizing compounds structurally distinct from Paclitaxel (Taxol(r)). Since epothilones are not a substrate for P-glycoprotein, are effective against Taxol-resistant cell lines, and are more soluble than Taxol, they are considered to be likely successors to Taxol. Unfortunately, the producing organism, *Sorangium cellulosum*, grows very slowly and produces only about 20 mg/L of epothilones. Furthermore, chemical synthesis of epothilones is impractical due to the complexity of the synthetic process. Overexpression of the epothilone gene cluster in *Streptomyces* has been accomplished, but yields of only 50-100 microgram/liter were obtained. In the PI's possession is a unique collection of *S. cellulosum* assembled from the 1950's through the early 1970's by Dr. John Peterson, a notable expert on *S. cellulosum*. Researchers worldwide acknowledge the uniqueness and scientific potential of this collection which currently consists of approximately 400 uncharacterized strains. In lieu of the promise epothilones hold as potential cancer therapeutic agents, and the possession of a historic *S. cellulosum* culture collection assembled by a noted expert, the broad, long-term goal of this project is to obtain epothilones in quantities suitable for evaluation on the chemical and clinical levels. To accomplish this goal, the specific aims of this project are: Aim 1) To screen a unique collection of approximately 400 uncharacterized strains of *S. cellulosum* assembled by Dr. John Peterson for production of epothilones; Aim 2) To investigate alternate growth conditions for *S. cellulosum* leading to increased production of epothilones, and Aim 3) To perform random mutagenesis on wild-type organisms to obtain overproducers of epothilone.

Grant: 1R01DA017293-01
Program Director: HILLERY, PAUL
Principal Investigator: JAVITCH, JONATHAN A MD
Title: ARCHAEOAL & BACTERIAL HOMOLOGS OF DOPAMINE TRANSPORTER
Institution: COLUMBIA UNIVERSITY HEALTH SCIENCES NEW YORK, NY
Project Period: 2003/09/30-2008/09/29

DESCRIPTION (provided by applicant): The dopamine transporter (DAT) is the major molecular target responsible for both the rewarding properties and abuse potential of cocaine and related psychostimulants. The homologous neurotransmitter transporters (NTs) for serotonin and norepinephrine, SERT and NET, are primary targets of antidepressant drugs. These integral membrane proteins couple the accumulation of neurotransmitter to the movement of sodium ions down their concentration gradient. Progress in the study of their molecular structure and transport mechanisms has been hampered by an inability to develop high-level expression systems for these proteins and the subsequent lack of sufficient functional, purified protein. Bacterial membrane proteins are generally more amenable to structural analysis and high-level expression than are their eukaryotic counterparts. We have recently identified an entire family of proteins in archaea and in bacteria (currently 73 proteins from 45 different organisms) that are homologous to DAT. The sequence identity to DAT for the most similar proteins is approximately 25 percent, making it very likely that they have a similar structure. Our strategy was to develop a high-level expression system with one or more of these proteins to obtain adequate amounts for direct structural studies. During the first 1.5 years of our Stage I Cutting-edge Basic Research Award (CEBRA), we have: a) cloned 17 of these genes from various bacterial and archaeal genomes, b) heterologously over-expressed 12 of these in the membrane of *E. coli*, c) shown that one of these gene products, TnaT, is a sodium-dependent tryptophan transporter, confirming that these genes encode proteins with functions similar to the NTs, and reaffirming their value as models for direct structural analysis, d) purified full-length TnaT from the membrane to near homogeneity in yields of approximately 0.6 mg/l culture, e) constructed a cysteine-less TnaT that is functional and expresses at near wild-type levels, and f) constructed strategically placed individual cysteine mutants that express and function normally. In this Stage II CEBRA proposal the specific aims are: 1) To identify residues within or very near the substrate binding site in TnaT, a sodium-dependent tryptophan transporter from *Symbiobacterium thermophilum*, using mass spectroscopic analysis of azido-tryptophan analogs photo-incorporated into TnaT. 2) To identify a drug-like inhibitor of TnaT by screening a combinatorial chemical library. 3) To identify solubilization conditions that preserve the structure and function of TnaT. 4) To establish conditions for functional reconstitution of TnaT into proteoliposomes. When these aims have been achieved, we will be in a position to choose a limited number of the bacterial transporters for use in crystallization trials as a step towards obtaining a high-resolution structure. Moreover, we will also be poised to pursue spectroscopic methods to dynamic structure. Success in either or both of these goals would revolutionize our structural understanding of the function of related human neurotransmitter transporters in away that is only a remote prospect through continued work on the eukaryotic transporters alone.

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Grant: 1R21DA016562-01
Program Director: THOMAS, DAVID D
Principal Investigator: CAUDLE, ROBERT M PHD
Title: Targeted Cholera Toxin for Treatment of Hyperalgesia
Institution: UNIVERSITY OF FLORIDA GAINESVILLE, FL
Project Period: 2003/05/01-2005/04/30

DESCRIPTION (provided by applicant): In a study conducted by the American Pain Society it was demonstrated that 9 percent of the United States population suffers from moderate to severe chronic pain. Current methods to treat chronic pain are in many instances not effective or produce a wide range of side effects that limit their utility. In this project we are going to take advantage of the recent discoveries that intrathecally administered cholera toxin blocks hyperalgesia and allodynia in rodent models of chronic pain, and that toxins can be directly targeted to nociceptive neurons in the spinal cord via the neurokinin 1 (NK1) receptor. We will conjugate the catalytic portion of cholera toxin to substance P in order to direct the cholera toxin to NK1 receptor expressing cells in the spinal cord. This conjugate will be tested for activity in NK1 expressing cell lines and in rodent models of chronic pain for its ability to stimulate cAMP production, suppress the expression of Gs g-proteins and to inhibit hyperalgesia and allodynia. The unique aspect of this conjugate is that cholera toxin will not kill the NK1 expressing cells in the spinal cord like saporin, diphtheria toxin and pseudomonas exotoxin, which have previously been used in NK1 receptor targeting strategies. Instead, cholera toxin will uncouple opioid receptors from Gs, thus enhancing their inhibitory actions, and will reduce the activity of Gs coupled receptor systems, which would further suppress nociceptive transmission. Thus, this project will be the first, to the best of our knowledge, to produce a therapeutic effect by directly manipulating g-protein function in an identified population of neurons in vivo. If successful, this project will produce a novel agent for the control of pain and will open up an entire new dimension in therapeutics by making g-proteins in specific cells the pharmacological target.

Grant: 2R01DC003685-06
Program Director: FREEMAN, NANCY
Principal Investigator: SCHACHT, JOCHEN H
Title: PROTECTION FROM AMINOGLYCOSIDE OTOTOXICITY
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 1998/05/01-2008/04/30

DESCRIPTION (provided by applicant): Aminoglycoside antibiotics remain the most commonly used antibiotics and the primary cause of preventable hearing loss worldwide. The impact of aminoglycoside ototoxicity has recently been aggravated by the global resurgence of tuberculosis and the increased occurrence of resistant bacteria necessitating multidrug regimens including aminoglycoside. Given the ten to 20% incidence of cochlear and vestibular disturbances associated with aminoglycoside treatment, this constitutes a major global health problem. The last decade has brought major advances in understanding the mechanisms of aminoglycoside action and designing interventions to prevent the ototoxic side effects. The proposed research is founded on past and recent discoveries from this laboratory that have led to the hypothesis of metal chelation and free radical formation by amino glycosides, and spurred by a first successful human trial demonstrating protection from gentamicin-induced hearing loss. The anticipated studies will follow new leads from preliminary experiments to delineate further the molecular mechanism of ototoxic action including the pathways of cell death and protection. Since we have recently established the adult mouse as a model for aminoglycoside ototoxicity we can combine biochemical and physiological investigations with the tools of molecular biology and the availability of mutant animals. In particular, our studies will 1. Characterize the potential contribution of lysosomal pathways to drug-induced hair cell death; 2. test the contribution of NF-kappaB mediated gene activation to cell survival; 3. analyze the regulation of the NF-kappaB pathway by signaling through phosphoinositide 3-kinase and Akt; 4. identify improved pharmacological protection based on an improved understanding of the mechanism of drug action. The results will define biochemical and molecular events involved in cell death and survival in aminoglycoside ototoxicity. The data may also help to understand other pathologies that are associated with oxidant stress, such as cisplatin ototoxicity, noise trauma and perhaps presbycusis. Optimized interventions to prevent aminoglycoside ototoxicity may serve as a basis for the translation of laboratory findings to the clinic. The attenuation or prevention of adverse effects of aminoglycosides will have far reaching implications for the continued but safe use of a family of drugs whose primary efficacy is unquestioned.

Grant: 1R01DC005659-01A1
Program Director: WATSON, BRACIE
Principal Investigator: POST, J CHRISTOPHER MD CLINICAL MEDICAL
SCIENCES, OTHER
Title: Pneumococcal Biofilms in Otitis Media
Institution: ALLEGHENY-SINGER RESEARCH INSTITUTE PITTSBURGH, PA
Project Period: 2003/04/01-2008/02/28

DESCRIPTION (provided by applicant): Otitis media (OM) is the most common reason that an ill child sees a health care provider, receives an antimicrobial or undergoes a general anesthetic. The overprescribing of antibiotics to treat OM is one of the major causes of antibiotic-resistant bacteria. Previous work from the Center for Genomic Sciences (CGS) has shown that culturally-negative middle-ear effusions actually contain bacterial DNA, mRNA and protein. These findings led to the rejection of the previous dogma, which held that chronic OM was a non-bacterial, inflammatory process, with the paradigm that OM is a mucosal biofilm disease. In conjunction with the Center for Biofilm Engineering, CGS has demonstrated that *Hemophilus influenza* can form biofilms in an animal model and in pediatric otorrhea. Biofilms are complex organization of bacteria covered with a protective exopolysaccharide matrix. In this state, bacteria are slow-growing and very resistant to antibiotics. In this application, we extend our work in biofilms by investigating *Streptococcus pneumoniae*, one of the most common bacteria associated with OM, and the most common respiratory pathogen. Our hypothesis is that understanding the differences in biofilm and planktonic gene expression will identify proteins that can serve either as immunogens for vaccine development, or targets for novel antimicrobial agents. Using state-of-the-art microarray technologies and a robust animal model of OM, the chinchilla, we propose to identify those genes that are necessary for *S. pneumoniae* to form a biofilm. Combining advances in imaging and molecular biology, we will directly examine the role of the differentially-regulated genes identified by the array technology. Delineating the molecular basis of biofilms will not only advance our understanding of OM, but of chronic infectious disease in general.

Grant: 1R01DC005855-01A1
Program Director: WATSON, BRACIE
Principal Investigator: GOLDSTEIN, RICHARD N PHD BIOLOGY NEC:BIOL
NEC-UNSPEC
Title: NTHi LPS: virulence factor and vaccine candidate
Institution: BOSTON UNIVERSITY MEDICAL CAMPUS BOSTON, MA
Project Period: 2003/08/01-2007/07/31

DESCRIPTION (provided by applicant): Nontypable *Haemophilus influenzae* (NTHi) is a major pathogen in otitis media. It appears to be especially prominent in children with recurrent episodes of acute otitis media (AOM). The broad, long-term objective of this proposal is to define the various roles of lipopolysaccharide (LPS) as a virulence factor in pathogenesis of AOM, with the ultimate goal of developing an LPS inner-core glycoconjugate vaccine against NTHi. Prior efforts in vaccine development have been limited by diversifying selective pressures on surface exposed epitopes of NTHi; however, the use of conserved LPS inner-core may circumvent this limitation. The first specific aim is to identify phenotypic and genotypic changes in NTHi LPS expression during transition from asymptomatic carriage to middle ear disease, in order to identify LPS glycoforms that may have potential as additional vaccine target candidates. Animal modeling in conjunction with mass spectrometry techniques and microarray technology should enable the identification of such critical LPS glycoforms. Secondly, interaction between newly resolved LPS glycoforms and host defense mechanisms will be assessed, by flow cytometry and ELISA techniques. The final aim is to determine whether truncated inner-core LPS oligosaccharide structures, when conjugated to a protein carrier, are immunogenic and protective against experimental OM due to both homologous and heterologous isolates of NTHi in a chinchilla animal model. This collaboration between Boston Univ. Medical School, Institute for Biological Sciences NRC-Canada and Oxford University Institute for Molecular Medicine brings together synergistic expertise in microbial genetics and population biology, immunology, LPS structural chemistry, and animal modeling.

Grant: 1R01DC005980-01
Program Director: WATSON, BRACIE
Principal Investigator: MUNSON, ROBERT S PHD MICROBIOLOGY, OTOLARYNGOLOGY
Title: Genomic Sequence of an Otitis Media Isolate of NTHi
Institution: CHILDREN'S RESEARCH INSTITUTE COLUMBUS, OH
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): At the current time, we have a poor understanding of how nontypeable *Haemophilus influenzae* (NTHi) cause otitis media (OM) in children. We hypothesize: (1) that there are as yet unidentified genes in NTHi that are important in the ability of this organism to cause OM; and (2) that there is a set of genes that are known but whose role in pathogenesis is uncertain or unsuspected. Under current funding, we have begun an in-depth study of NTHi strain 86-028NP pathogenesis in the chinchilla models of OM. Specifically, we have employed differential fluorescence induction using promoter probe constructs; and signature tag mutagenesis to identify strain 86-028NP genes differentially expressed in NTHi and/or genes obligatorily required at one or more stages of the infectious process. We have also sequenced, to 3-fold coverage, the genome of NTHi strain 86-028NP. Some of the genes we identified in NTHi have homology to genes present in the published *H. influenzae* strain Rd genome. Sequences with no homology to Rd genes but with homology to known genes of other organisms, or unique genes which have no homology to previously identified genes, also have been identified. Although the genome information has been useful and informative, the assembly currently contains 576 contigs, many with regions of low coverage. The current contig data is available on our web site at www.microbial-pathogenesis.org. A global analysis of the current assembly indicates that the gene content and order are similar to that seen in strain Rd. A more detailed analysis reveals that there are a substantial number of genes not previously seen in the Pasteurellaceae and some regions where the gene content and order is different than seen in strain Rd. Thus, the current data suggest that the strain 86-028NP genome will contain a complex mosaic of Rd and non-Rd like features, features that may be important but are not completely discernable from the available data. The tremendous interest in vaccine development for NTHi disease, the increase in our understanding of the pathogenesis of NTHi disease and the knowledge that will be gained from the comparative genomics of different members of the Pasteurellaceae family makes it imperative that we have the complete genome sequence of at least one pathogenic NTHi strain. The National Institute on Deafness and Other Communication Disorders (NIDCD) has been extremely supportive of the functional studies of strain 86-028NP (R01DC03915 from NIDCD/NIH, Lauren Bakaletz, PI) as well as the funding to obtain the 3-fold genome sequence coverage of this economically important pathogen (supplement to R01DC03915 from NIDCD/NIH). We propose to close and annotate the genome of nontypeable *H. influenzae* strain 86-028.

Grant: 1R01DC006279-01

Program Director: WATSON, BRACIE

Principal Investigator: WASSERMAN, STEPHEN I MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC

Title: Mast Cells and innate immunity in otitis media

Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA

Project Period: 2003/07/01-2008/06/30

DESCRIPTION (provided by applicant): Otitis media is a major health problem causing substantial morbidity and resulting in substantial health care expenditures. The causes and treatment of this disorder remain incompletely understood. Eustachian tube obstruction, viral and subsequent bacterial infection and immunity have each been suggested to contribute to the etiopathophysiology of this disorder. Recent work in our laboratories has indicated that concurrent mast cell activation and bacterial infection can synergistically interact to induce strong inflammatory changes in the middle ear. These preliminary data, as well as prior research in the middle ear and other systems, suggest that the mast cell may play an important role in the innate and cognate defense of the middle ear, and may also contribute to otitis media pathogenesis. In this proposal Drs. S. Wasserman, A. Ryan and D. Broide propose a series of integrated experiments employing genetically modified mice to validate this hypothesis and to elucidate the mechanism(s) by which this synergistic interaction occurs. Aim 1 of this proposal will define the synergistic interaction utilizing mast cell deficient mice, with and without, reconstitution of their middle ear mast cells. Aim 2 will examine the mechanisms by which bacterial products enhance mast cell mediated synergistic inflammation by exploring the Toll-like receptor (TLR) pathways of mast cells. In these experiments TLR 2, 4, and 9 deficient mast cells will be used to re-constitute middle ear mast cell populations of mast cell deficient mice and the effect of bacterial/mast cell interactions defined. MyD88 deficient mice, defective in all TLR signaling will be used to define potential redundancies in these responses. Aim 3 will re-constitute middle ear mast cells with mast cell populations obtained from mice which are deficient in one or more mast cell mediators including histamine, leukotriene and tumor necrosis factor alpha to define the mediator(s) responsible for mast cell enhancement of inflammation induced in the presence of bacteria. Aim 4 will elucidate the leukocyte/endothelial mechanisms by which mast cell] bacterial interactions enhance inflammation, by direct observations of leukocyte behavior in genetically modified animals. Together these studies will expand our understanding of the role of mast cells in otitis media and may identify new targets for therapeutic intervention.

Grant: 1R21DC006084-01
Program Director: FREEMAN, NANCY
Principal Investigator: STEYGER, PETER S PHD
Title: Functional similarities in renal and cochlear epithelia
Institution: OREGON HEALTH & SCIENCE UNIVERSITY PORTLAND, OR
Project Period: 2003/03/15-2005/02/28

DESCRIPTION (provided by applicant): Aminoglycosides are clinically essential for treating life-threatening Gram-negative bacterial infections, e.g., meningitis, and in preventing infection in burns and premature babies. However, there is a high incidence of ototoxicity and sensory hair cell death. Sensitive hair cell function is dependent on a structurally and physiologically intact ear. Thus, studies to determine mechanisms of ototoxicity have used intact animals, or inner ear explants excised from animals. There are a number of functional, toxicological, and pharmacological similarities between inner ear cells and cells of the kidney tubules. To exploit these similarities, we have developed cloned sub-lines of currently-available kidney cell lines to determine their validity as in vitro models to study the mechanisms of aminoglycoside transport and toxicity in the inner ear. Using the aminoglycoside gentamicin, we propose to use cloned kidney cells to study two distinct aspects of aminoglycoside ototoxicity: 1) Like hair cells, proximal tubule epithelial cells of the kidney are among the few cell types that are toxicologically sensitive to clinical doses of aminoglycosides. These cell types also share pharmacological sensitivities to several other drugs. We will use a clone of OK cells, a proximal kidney tubule line, to determine the feasibility of using proximal tubule cells as valid in vitro models of aminoglycoside uptake and toxicity by hair cells. 2) Little work has been done to determine the mechanism of drug entry into the endolymph. Research suggests that hair cell uptake of aminoglycosides is across the apical membrane that is exposed only to the highly-regulated endolymph. Thus, pathways of drug entry into the endolymph will be highly-potent sites of intervention to prevent ototoxicity during treatment. The various epithelia surrounding the endolymph are cumbersome to acquire as explants in the quantity and proper configurations to facilitate direct examination of aminoglycoside transit across these epithelia. Therefore, we have developed a kidney distal tubule cell line to use as a model for aminoglycoside transport across the various epithelia enclosing the endolymph compartment. Distal tubule cells share several characteristics with inner ear epithelia such as the marginal cells of the stria vascularis. We will use a clone of MDCK cells, a distal tubule line, to determine the feasibility of using distal tubule cells as valid in vitro models for aminoglycoside transport across the epithelia enclosing the scala media. Results obtained from both studies will periodically be compared with animal models for confirmation. Preliminary data obtained with use of our cloned kidney cell lines have already dramatically changed our understanding of aminoglycoside entry into, distribution within, and transit across epithelia. Both of these in vitro models should provide powerful tools for finding co-therapeutics to reduce or eliminate ototoxicity during aminoglycoside treatment, blocking both toxic events in the hair cells and drug access to the endolymph and, thus, the hair cells.

Grant: 1R21DC006260-01
Program Director: WATSON, BRACIE
Principal Investigator: PETTIGREW, MELINDA M BA
Title: Gene Discovery for Pneumococcal Otitis Media
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 2003/07/01-2006/06/30

DESCRIPTION (provided by applicant): Otitis media (OM) is the most common bacterial infection in the United States among young children for which medical care is sought, and *Streptococcus pneumoniae* strains are the leading cause of bacterial OM. These gram-positive diplococci colonize the respiratory tract in up to 50% of healthy children and only cause disease under special circumstances. Recent genomic sequencing projects and advancements in molecular biology have resulted in the identification of numerous putative virulence factors. These factors have mainly been studied in the context of invasive pneumococcal diseases such as pneumonia, or have been studied in one or a few laboratory strains. While more virulence factors remain to be discovered, the new challenge is to identify which of these many factors warrant further study, to link these factors specifically to OM pathogenesis, and to estimate the relative importance of these virulence factors among the *S. pneumoniae* strains in circulation. This project is built on the observation that *S. pneumoniae* strains differ in their ability to cause disease, and that these differences are likely due to genetic differences between strains that extend beyond the polysaccharide capsule. The goal of this project is to identify genes associated with pneumococcal OM and to evaluate the relative frequency of pneumococcal virulence genes among a collection of isolates obtained from healthy children and children with clinical disease. A four step interdisciplinary approach utilizing techniques of molecular biology and epidemiology will include: 1. Selection of *S. pneumoniae* strains for genomic subtraction with the highest potential to identify genes associated with OM. 2. Identification of DNA sequences (sPCR fragments) unique to strains causing OM (tester strains) and absent in strains from healthy carriers (driver strains) using genomic subtraction. 3. Epidemiologic screening of a large collection of isolates from healthy children and children with OM, meningitis, pneumonia, or bacteremia using sPCR fragments. 4. Identification of genes associated with sPCR fragments important for OM pathogenesis and description of their biological and clinical characteristics. Discovery of additional factors involved in streptococcal OM will facilitate the development of new strategies for the control and prevention of this important disease.

Grant: 2R01DE007559-19A1
Program Director: SHUM, LILLIAN
Principal Investigator: GRAVES, DANA T
Title: Bone-derived Cells Produce a Chemotactic Factor
Institution: BOSTON UNIVERSITY MEDICAL CAMPUS BOSTON, MA
Project Period: 1987/09/01-2007/03/31

DESCRIPTION (provided by applicant): Periodontal disease is characterized by the loss of connective tissue and alveolar bone. The loss of these tissues is initiated by oral bacterial that induce an inflammatory cascade leading to the destructive events. While the molecular actions that result in lysis of connective tissue matrix and bone resorption have been well defined, it is not known why the repair mechanisms present in both tissues are not effective in restoring the damage which occurs. Thus, the two clinically significant events in periodontitis, net loss of attachment and alveolar bone, can be linked to inadequate repair after bacteria-induced damage. That one of the characteristic changes that occurs in episodes of recent periodontal breakdown is the loss of fibroblasts suggests that apoptosis may be an important antecedent to the net tissue loss. Preliminary data indicate that the super-periosteal injection of *P. gingivalis*, a prominent periodontal pathogen, into the scalp causes marked inflammation, destruction of connective tissue matrix followed by infiltration and proliferation of fibroblastic cells. Likewise, bone resorption occurs followed by the formation of new woven bone. We will use this model to examine the events that occur following *P. gingivalis* infection focusing on apoptosis and repair. The goal for the proposed studies is to test the hypothesis that periodontal pathogens induce apoptosis of critical cells and thereby impede normal repair of bone and connective tissue matrix. We propose that induced apoptosis is mediated via TNF-induced caspase activity. These studies will provide new insight into the mechanisms for a net loss of periodontal tissues taking an approach that has not been previously applied to this problem.

Grant: 2R01DE009760-11A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: FIVES-TAYLOR, PAULA M PHD
MICROBIOLOGY:MICROBIO
OGY-UNSPEC
Title: Invasion and Adhesion of Periopathogens
Institution: UNIVERSITY OF VERMONT & ST AGRIC Burlington, VT
COLLEGE
Project Period: 1992/03/01-2007/11/30

DESCRIPTION (provided by applicant): Actinobacillus actinomycetemcomitans (Aa) is a non-motile, Gram-negative facultative coccobacillus, which colonizes the human oral cavity and upper respiratory tract. Overwhelming microbial, immunological and clinical evidence implicates Aa in the pathogenesis of localized juvenile periodontitis and cases of rapid and refractory periodontal disease. This pathogen has been associated with other serious human infections such as endocarditis, soft tissue abscesses, and more recently cardiovascular disease. The periodontium has been implicated as the reservoir of these extraoral infections, although little is known about the mechanisms this pathogen utilizes to infiltrate and disseminate in tissues. The invasion into epithelial cells in vitro is well established and this periodontopathogen has been localized inside epithelial cells and found in sub epithelial cell layers in tissue biopsy from infected individuals. Our laboratory has demonstrated that Aa utilizes microtubules for intracellular trafficking and exit from epithelial cells. The data suggest that microtubules play a critical role in the spread and movement of Aa and provide the first evidence that host cell dispersion of an intracellular pathogen may involve the usurpation of microtubules. To further understand this unique interaction, we propose to 1) identify and characterize the bacterial macromolecule(s) that are involved in the interaction with microtubules and 2) characterize the component(s) of the epithelial cell microtubule asters that interact with Aa. The identification of these components will be important in understanding the processes that this pathogen uses to survive in the oral cavity and disperse into deeper tissues.

Grant: 2R01DE010058-10A2
Program Director: MANGAN, DENNIS F.
Principal Investigator: BANAS, JEFFREY A
Title: Glucan Binding Proteins of Oral Streptococci
Institution: ALBANY MEDICAL COLLEGE OF UNION ALBANY, NY
UNIV
Project Period: 1992/08/01-2008/04/30

DESCRIPTION: Streptococcus mutans possesses three distinct glucosyltransferases (GTFs) and at least three different nonenzymatic glucan binding proteins (GBPs). The precise contributions of each GTF and GBP to plaque development are unknown. Recently we showed that the loss of a glucan-binding protein (GbpA), accomplished through allelic replacement, dramatically altered the architecture of the biofilm formed by cultures of S. mutans. Based on these data we engineered strains with inactivation in genes encoding other extracellular proteins. The loss of GbpC, FruA, or P1 also resulted in changes in the biofilm architecture. To explain these observations we posit that the loss of an extracellular protein may result in specific physical and/or biochemical changes to the organism, or to its immediate environment that affect the structure of the mature biofilm formed by that organism. The Specific Aims of this application are designed to test these possibilities. Since work with the GbpA has progressed the furthest, investigations of specific GbpA properties are also included in the Aims. Aim 1) Examine the physical properties of the knockout strains including hydrophobicity, surface charge, and their interactions with cations such as calcium which are important in the development of a biofilm. Aim 2) Use a flow chamber to examine the events associated with the initial attachment of the knockout strains to the substratum and correlate the results with the structure of the mature biofilms. Aim 3) Examine how the ratio of GbpA to glucan synthesis correlates with the structure of the biofilm. Aim 4) Delete or replace the amino terminal domain (non glucan-binding domain) of GbpA and examine how this change affects the phenotypic properties of Streptococcus mutans compared to the wild-type and GbpA knockout strains. Aim 5) Express phenotypic properties of formation. Aim 6) Utilize architecture influence gene GbpA in the heterologous host Streptococcus gordonii and examine the organism in the context of sucrose-dependent adhesion and biofilm microarrays to determine how the loss of extracellular proteins and biofilm expression.

Grant: 2R01DE010729-08
Program Director: MANGAN, DENNIS F.
Principal Investigator: DEMUTH, DONALD R PHD
Title: Control of A actinomycetemcomitans Leukotoxin Expression
Institution: UNIVERSITY OF LOUISVILLE LOUISVILLE, KY
Project Period: 1994/04/01-2008/04/30

DESCRIPTION: *A. actinomycetemcomitans* is associated with human periodontal disease and expresses a leukotoxin (ItX) that kills human cells that are required for the immune response against infection. Most *A. actinomycetemcomitans* strains express low levels of this toxin, but some strains contain alterations in the ltx promoter and exhibit a hyper-leukotoxic phenotype. These strains exhibit altered regulation of the ltx genes and are associated with localized juvenile periodontitis. We will determine how the ltx genes are regulated in minimally leukotoxic organisms and how the regulatory processes are altered in hyper-leukotoxic organisms. We have identified a c/s-acting negative regulator of toxin expression that interacts with a trans-acting protein. This protein will be isolated and we will determine how this interaction reduces ltx expression. We will also determine if the acquisition of an insertion element (IS) leads to increased toxin expression by displacing this c/s-acting negative regulator upstream from the ltx genes. We will also determine if IS introduces an outwardly directed promoter that transcribes the ltx genes. We have also shown that ltx expression is induced during anaerobic growth. A c/s-acting sequence and a trans-acting polypeptide that may play a role in anaerobic regulation have been identified. We will determine if this sequence functions as an UP element that interacts with the ltx-subunit of RNA polymerase. Finally, the ltx operon possesses a fifth gene, orphan, that resides upstream from IXC. Frame shift mutations in orfA reduce ltx expression. Therefore, we will determine if the OrfA polypeptide is involved in the regulation of ltx expression and whether a naturally occurring deletion in orfA leads to the hyper-leukotoxic phenotype. It is clear that the leukotoxin is an important virulence determinant of *A. actinomycetemcomitans* and thus it is important to understand the mechanisms that lead to the hyper-leukotoxic phenotype. These studies will determine how ltx expression is regulated in *A. actinomycetemcomitans* and will identify mechanisms that contribute to hyper-expression of the toxin. The cis- and/or transacting components that are found to differ in ltx hyper-expressing strains may represent new diagnostic targets to detect hyper-virulent *A. Actinomycetemcomitans*. It is also possible that the regulatory processes themselves may represent targets that can be exploited for the development of local or systemic therapies Aimed at affecting leukotoxin expression in vivo.

Grant: 2R01DE010861-06A2
Program Director: BHARGAVA, SANGEETA
Principal Investigator: ZADEH, HOMAYOUN H DDS
Title: Subversion T Cell Response by A. actinomycetemcomitans
Institution: UNIVERSITY OF SOUTHERN CALIFORNIA LOS ANGELES, CA
Project Period: 1996/03/15-2008/05/31

DESCRIPTION (provided by applicant): Aa is a major pathogen implicated in several forms of periodontal diseases, and non-oral infections. Despite extensive investigation, the exact mechanism of pathogenicity of this microorganism remains obscure, though it is believed that the host mediates much of it. There is evidence that this bacterium uses a number of mechanisms to evade the host response. Recent studies in our laboratory have demonstrated that Aa induces a very potent T cell activation response. While up to two-third of all T cells are activated, they exhibit impaired cytokine expression and the majority undergoes apoptosis. The few T cells that do express cytokines, predominantly express IL-10, which has immunosuppressive effects. Many periodontitis patients fail to mount an effective antibody response against periodontal pathogens. Based on data, we have posited that Aa interferes with antigen-specific T cell response by large-scale antigen nonspecific activation. The activated cells include regulatory T cells that suppress the antigen-specific T cell response. We further posit that the Aa cytotoxins kill the majority of T cells by apoptosis. To investigate our hypothesis, we have proposed Specific Aims to: 1) characterize the cellular and molecular mediators of T cell apoptosis; 2) examine the nature of the suppression induced by regulatory T cells and 3) identify and characterize the functional attributes of Aa-specific T cells found in periodontitis sites. The characterization of Aa-mediated immunosuppression will not only aid in understanding of the mechanism of pathogenicity of this organism, but it may offer additional experimental tools for manipulating the T cell response. There is currently great need for developing immunosuppressive tools for therapy of autoimmune diseases and prevention of graft rejection.

Grant: 2R01DE012305-06A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: LALLY, EDWARD T DMD
Title: A. actinomycetemcomitans Leukotoxin Induced Cell Death
Institution: UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA
Project Period: 1997/07/01-2008/04/30

DESCRIPTION: Actinobacillus actinomycetemcomitans is a facultative anaerobe that has been implicated in a variety of periodontal diseases. Its presence is most closely associated with localized juvenile periodontitis (LJP) where it is believed to be the etiologic agent. The role this organism plays in other periodontal diseases, however, is not entirely clear. While A. actinomycetemcomitans produces a number of virulence factors, a leukotoxin (Ltx) appears to be most associated with enabling this organism to establish an ecological niche and thereby achieve its pathogenic potential. This concept is supported by an increasing number of clinical studies that demonstrate a reproducible ability to culture strains with the high leukotoxic phenotype from patients with active LJP. The mechanism by which A. actinomycetemcomitans Ltx kills target cells is ambiguous since two mechanisms of eukaryotic cell death have been identified: necrosis and apoptosis. High concentrations ($> 10^4$ M) of Ltx induce very rapid target cell death with little morphologic or cytometric evidence of apoptosis. Nevertheless, cells exposed to low concentrations of Ltx exhibit an alteration in mitochondrial transmembrane permeability that is followed by: 1) dissipation of the mitochondrial transmembrane potential, 2) release of apoptosis-inducing factors, 3) generation of reactive-oxygen radicals, and 4) depletion of ATP stores. The earliest change that we have observed following the addition of Ltx to susceptible cells is a rise in intracellular calcium $[Ca^{2+}]$; which is both immediate (within 10 sec) and sustained. The Ltx-induced Ca^{2+} influx and apoptosis exhibit an identical dose-dependence and point to a mechanistic connection linking of Ca^{2+} influx and the onset of apoptosis. The ability of a mAb to neutralize the cytolytic activity of the Ltx and to inhibit the $[Ca^{2+}]$ changes are a strong indication that the reaction begins with Ltx. The proposed investigations are designed to increase our understanding of Ltx cytotoxicity by: 1) exploring early steps that result in Ltx-induced $[Ca^{2+}]$ increases in target cells; 2) identifying the origin(s) of the increased $[Ca^{2+}]$ seen in Ltx-treated cells; 3) defining effects Ltx-induced $[Ca^{2+}]$ increases have on cellular organelles and 4) characterizing the function of the endoplasmic reticulum in Ltx-treated cells.

Grant: 2R01DE012593-04A2
Program Director: MANGAN, DENNIS F.
Principal Investigator: DIRIENZO, JOSEPH M
Title: Study of Actinobacillus Actinomycetemcomitans Virulence
Institution: UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA
Project Period: 1999/08/01-2008/03/31

DESCRIPTION: The periodontal pathogen, *Actinobacillus actinomycetemcomitans*, expresses several complex multi-gene toxin systems that promote the negative interaction of this bacterium with human cells. These systems include genes required for invasion and genetic loci for a leukotoxin and a cytolethal-distending toxin (CDT). This impressive and extensive repertoire of potential virulence factors has not been found in other oral microbial pathogens. The focus of this application is a continuation of our studies of the CDT of *A. Actinomycetemcomitans* Y4. This toxin is composed of three gene products which self-assemble to form a tripartite complex or holotoxin. The active subunit of the holotoxin is a nuclease, which is functionally related to mammalian DNase I. The DNase I-like protein targets the nucleus of most eukaryotic cells where it causes extensive DNA fragmentation and chromatin damage. These activities lead to growth arrest at the end of the G2 phase of the growth cycle. The major objectives of this application are to understand how the various subunits of the CDT function to produce a biologically active toxin, to define the interactions of the CDT with cell lines that are relevant to the integrity of the human oral cavity and to isolate and use CHO cell mutants to study cytotoxicity. The Specific Aims are: (i) to determine the interrelationships of the *cdtA*, *cdtB* and *cdtC* gene products, (ii) to determine the differential effects of the CDT on cultured oral epithelial cells and fibroblasts, and (iii) to use CHO cell mutants to examine and characterize specific roles of *cdt* gene products in the various stages of intoxication. Natively assembled recombinant holotoxin and holotoxin assembled in vitro, from purified recombinant Cdt proteins, will be used in cytotoxicity and binding experiments to examine the functions of the subunits. The differential effects of the CDT on a recently immortalized human oral epithelial cell line and human periodontal ligament fibroblasts will be examined to define the specificity and host range of the CDT. In a novel approach, CHO cell mutants resistant to the activities of the various CDT subunits will be isolated and used to segregate the subunit activities for study. The long-term goal of our study is to gain insight into the role of the *Actinobacillus actinomycetemcomitans* CDT in the perturbation of normal cell functions that are important for maintaining the healthy status of the human oral cavity.

Grant: 2R01DE013230-04A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: CVITKOVITCH, DENNIS G PHD
Title: The competence regulon in *Streptococcus mutans* biofilms
Institution: UNIVERSITY OF TORONTO TORONTO, ON
Project Period: 1999/09/01-2006/02/28

DESCRIPTION: *Streptococcus mutans* resides in the biofilm of Dental plaque where it produces acid from dietary carbohydrate to cause caries. *S. mutans* uses a quorum-sensing signaling system in biofilms to activate genetic competence, acid tolerance and influence biofilm architecture. This application addresses mechanisms by which cell-cell and environmental signals activate this biofilm phenotype. *S. mutans* quorum-sensing system is encoded by the *comCDE* genes that encode a competence-stimulating peptide (CSP) precursor, a histidine kinase and a response regulator. We expect to answer: 1) Which of the genes activated by CSP are involved in biofilm formation? 2) How is this pathway regulated? 3) Can we inhibit this pathway to attenuate the biofilm phenotype of *S. mutans*? 4) Do other environmental signals activate the biofilm phenotype? Differential display (ddPCR) and 2D gel electrophoresis will be used to identify genes and proteins that are activated by the CSP. An isogenic *comC* (CSP-deficient) mutant will be used to decipher this regulon. Its ddPCR and 2D expression profiles with and without exogenous synthetic CSP will be compared. Genes encoding products with altered expression will be cloned and mutated using a novel allelic exchange technique; the mutants genetic competence will be tested. Cell segregation and the 3D architecture of the mutant biofilms will be assessed by SEM and Confocal Scanning Laser Microscopy (CSLM). Reporter gene fusions (*gfp*, *lacZ* and *luc*) constructed in these genes will be used to measure gene expression temporally in response to CSP using fluorometric and luminetric analysis and CSLM to give insight into how the CSP signaling cascade functions to influence biofilm phenotype in real time. CSP analogs will be tested for their inhibition of the signal transduction process. *S. mutans* also has two component signal transduction systems (TCSTS) to sense environmental signals. Bioinformatic analyses have identified 13 TCSTS in *S. mutans*, and mutants defective in their individual genes were constructed. Using a robotic growth monitor, the mutants will be examined for the ability to grow in the presence of environmental stresses: acid, salts, peroxide, SDS, and high sugar concentration to elucidate the function of the TCSTS. These experiments will give insight into the signaling mechanisms used by *S. mutans* and potentially other biofilm-forming bacteria.

Grant: 1R01DE014372-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: HACKETT, MURRAY PHD
Title: Proteomics of *P. gingivalis* invasion of epithelial cells
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2003/09/01-2008/06/30

Porphyromonas gingivalis, a Gram-negative anaerobe, is a major etiologic agent of severe adult periodontitis. *P. gingivalis* possesses a number of virulence factors including the ability to invade the epithelial cells of the gingiva. In primary cultures of human gingival epithelial cells (GECs) the internal bacteria rapidly locate in the cytoplasm, predominantly in the perinuclear area, where they can replicate and reach a high density. The molecules of *P. gingivalis* that direct these events have yet to be determined. GECs are being used as a model system to study host-pathogen interactions involved in human periodontal disease. We propose to take a comprehensive, proteomics based approach to the study of *P. gingivalis* invasion and virulence by examining changes in global protein expression during invasion for the pathogen. For a select group of proteins, timecourse measurements of protein expression by mass spectrometry will be compared to mRNA levels measured using semi-quantitative real time reverse transcriptase polymerase chain reaction (RT-PCR) and Northern blot analyses. Expected changes in *P. gingivalis* hydrophobic membrane bound proteins will be assayed as well, using non-aqueous reversed-phase HPLC technology developed at the University of Washington and (or) the MudPIT approach (Multidimensional Protein Identification Technology) of the Yates laboratory, both of which expand the range of proteins that can be analyzed beyond what can be done with the current state-of-the-art in 2D gel electrophoresis in terms of isoelectric point and hydrophobicity. The transcription analysis will be complemented by our ability to map observed proteins to the *P. gingivalis* genome in a semi-automated fashion directly using mass spectral fragmentation data. The data sets from these experiments will be used to gain insights into the precise molecular determinants of *P. gingivalis* invasiveness, that will aid in the characterization of potential targets for therapeutic agents that could serve to inhibit the transformation of *P. gingivalis* from a harmless commensal colonizer into a highly invasive pathogen.

Grant: 1R01DE014604-01A2
Program Director: MANGAN, DENNIS F.
Principal Investigator: PIGGOT, PATRICK PHD
Title: Stationary Phase Behavior in Oral Streptococci
Institution: TEMPLE UNIVERSITY PHILADELPHIA, PA
Project Period: 2003/07/01-2007/04/30

DESCRIPTION: *Streptococcus mutans* is the primary agent of Dental caries. We think that stationary-phase bacteria are critical to the behavior and survival of *S. mutans* in the Dental plaque biofilm, and hence to its role in Dental caries. They are surely a critical part of the feast-or-famine lifestyle of *S. mutans*, as famine causes entry into the stationary phase. Yet, most studies of *S. mutans* have been of exponentially growing bacteria. We propose to test the general hypothesis that stationary-phase bacteria in biofilms behave differently from vegetative bacteria and have an important role in determining the properties of mature biofilms, and in particular in their persistence. We propose using single-species biofilms formed in flow cells to test this hypothesis. We propose to test how well stationary-phase bacteria survive prolonged carbon-source starvation in biofilms and how they respond to nutrient restoration and acid shock. We will test the effect of different treatments on the shedding of bacteria from stationary-phase biofilms, and test how effectively the shed bacteria can initiate secondary biofilm formation in a second flow cell. We propose to use the fluorescing protein GFPmut3b* as a probe to locate bacteria expressing known stationary-phase-specific genes and those expressing vegetative-phase specific genes. This will help clarify the difference in properties between the two bacterial types. We will screen for new stationary-phase expressed genes. These genes will enable testing if there are different types of stationary-phase bacteria with different resistances and different responses to nutrient. They will give an indication of the sorts of function that are expressed during stationary phase. We propose to test the roles of these genes. We propose to identify and characterize the regulators of stationary phase gene expression. These studies should lead to a better understanding of how *S. mutans* in mature Dental plaque biofilm persists and responds to fluctuations in its environment.

Grant: 1R01DE014714-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: WALKER, CLAY B PHD
Title: ANTIBIOTIC RESISTANCE IN A SUBGINGIVAL BIOFILM MODEL
Institution: UNIVERSITY OF FLORIDA GAINESVILLE, FL
Project Period: 2003/08/01-2007/04/30

DESCRIPTION (provided by applicant): We have developed biofilm models of the subgingival plaque on various supports (hydroxyapatite, nitrocellulose membranes, and glass) that are relative simple in concept, but yield reproducible results and mimic the complexity and the diversity of the subgingival microflora. These models appear to be useful for studying the development, maturation, and ultrastructure of the subgingival plaque. The hydroxyapatite model will be used to study the development of the subgingival microflora and the effects that antibiotics exert on the bacteria present. The biofilms grown on the membrane and glass supports will be used to microscopically study the ultrastructure of the biofilms by transmission electron microscopy and confocal laser scanning microscopy. We propose, after refining and validating the model, to use this model to determine the effects of antibiotics on biofilms grown from subgingival plaque collected from sites with periodontitis. These data will be compared to the effect of antibiotics on planktonic cultures of the predominant bacterial isolates present in the biofilm to determine differences in antibiotic susceptibilities in a biofilm relative to planktonic broth cultures. The second objective is to determine the effect of antibiotics, at concentrations obtained therapeutically, on developing biofilms and on a mature biofilms. This will provide a realistic approximation of the effect that antibiotics have on the periodontal flora when used as adjuncts to conventional scaling and root planing. The final specific aim will examine the transfer of antibiotic resistant determinants in the biofilm model. The horizontal transfer of tetQ, a major tetracycline-resistant determinant in the oral flora, will be investigated both within the normal bio-community associated with the subgingival plaque and with exogenous tetracycline-resistant bacteria that are not normal inhabitants of the subgingival community but may occur as transients in this community. We believe the information derived from the experiments described will provide a better understanding of the subgingival flora and the effects that antibiotics may have on this flora.

Grant: 1R01DE014749-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: SHARMA, ASHU PHD
Title: B.forsythus BsPA protein: role in virulence
Institution: STATE UNIVERSITY OF NEW YORK AT Amherst, NY
BUFFALO
Project Period: 2003/04/01-2006/12/31

DESCRIPTION: *Bacteroides forsythus* is a Gram-negative oral anaerobe implicated in the development of periodontal disease pathogenesis. Although, very little is known about the virulence factors of this organism, based on our recent in vitro and in vivo studies, a surface-associated 98-kDa protein (BspA) has been suggested as a virulence factor. The BspA protein contains homologous sequences belonging to the leucine-rich repeat motif family (LRR), and to motifs belonging to the immunoglobulin superfamily (Ig-SF). In vitro, the BspA protein binds to extracellular matrix components fibronectin and fibrinogen, and to epithelial cells, and induces release of proinflammatory cytokines from monocytic cells. Further, a mutant of *B. forsythus* defective in BspA expression constructed in our laboratory has been found to be significantly attenuated in its ability to bind to fibronectin, fibrinogen, and epithelial cells. The studies proposed here will address the hypotheses that LRRs and IgSF domains are critical for host cell interactions via binding to specific cellular receptors, and that the BspA protein plays important roles in pathogenesis via mediating bacterial colonization and triggering of host cellular responses, such as release of cytokines and other mediators. The experimental design will include: 1) studies to determine the specific BspA-domains involved in host cell (epithelial and monocytic cells) interactions (Specific Aim 1a), and investigate intracellular signaling events resulting from BspA binding (Aim 1b); 2) biochemical characterization of epithelial (Aim 2a) and monocyte receptors (Aim 2b) that bind BspA protein; and 3) assessment of the in vivo role of BspA protein as judged by studies in a mouse model of periodontal disease (Aim 3a), and by evaluating the host immune response against the BspA protein in patients with a history of periodontitis (Aim 3b). The findings will be important in determining the roles of BspA protein, and the underlying contribution of its domains in bacterial pathogenesis. The studies will also be critical from a proteomic standpoint in defining the roles of LRR and Ig-like signatures found in other bacterial proteins in general. In the long term, understanding the basic mechanisms of the BspA-mediated pathogenesis of *B. forsythus* will be vital in developing intervention strategies against periodontal disease.

Grant: 1R01DE014757-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: QI, FENGXIA PHD
Title: S. mutans: Its mutacin Antibiotic
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 2003/03/03-2007/11/30

DESCRIPTION: Streptococcus mutans is the major etiologic agent responsible for Dental caries, the most prevalent disease in the developed and developing countries. Most clinical isolates of S. mutans produce antimicrobial peptides called mutacins. Mutacins are active against a wide spectrum of Gram-positive bacteria including pathogens and oral commensals. Thus, mutacin production by S. mutans may play a double role: it provides the producing strain with a competitive edge in gaining dominance in the Dental plaque, leading to Dental caries. On the other hand, it may protect the human host from Gram-positive bacterial infections. The proposed research Aims to study the genetic, biochemical and biological aspects of mutacin biogenesis and regulation with the following approaches: Aim 1, to characterize the trans-acting factors for mutacin gene regulation. Aim 2, to characterize the cis-acting factors that regulate mutacin gene expression. Aim 3, to determine the structure/function of the mutacin molecule and improve the properties of mutacins by genetic engineering. Aim 4, to enhance mutacin biosynthesis and improve fermentation conditions. The proposed research will have a significant impact on two fronts. The first is the alarming surge in resistance to the existing battery of antibiotics by emerging and existing pathogens, and the increasing threat of bioterrorist attack using genetically engineered pathogens resistant to all existing antibiotics. These threats underpin the importance and urgency of finding unconventional antibiotics, to which resistance has not been developed. The second front is understanding the mechanism of gene regulation for mutacin production in S. mutans. This knowledge will help design effective control measures to curb the growth and virulence of S. mutans in the Dental plaque.

Grant: 1R01DE014774-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: GIBSON, FRANK C PHD
Title: P. gingivalis Capsule in Cell Inflammation
Institution: BOSTON MEDICAL CENTER BOSTON, MA
Project Period: 2003/05/01-2008/01/31

DESCRIPTION: Porphyromonas gingivalis, a Gram-negative, anaerobic, oral pathogen is the primary etiologic agent of adult periodontal disease. Recent reports indicate that encapsulated P. gingivalis are more virulent than unencapsulated strains. Nevertheless, the precise role of P. gingivalis capsular polysaccharide in disease pathogenesis, and the mechanism by which this virulence factor acts, are largely unknown. We posit that the capsular polysaccharide of Porphyromonas gingivalis promotes a cellular inflammatory response characteristic of P. gingivalis infection, and contributes to the pathology observed in P. gingivalis-mediated adult periodontal disease. Our preliminary, in vitro studies demonstrate that this antigen binds to host cells and stimulates an innate immune response, which is permissive for PMN chemotaxis. In vivo observations confirmed our in vitro data, and demonstrate that purified P. gingivalis capsular polysaccharide stimulates a host inflammatory cell response, that mimics live, P. gingivalis whole cell challenge. Three Specific Aims are proposed: 1): To define the binding kinetics of purified Porphyromonas gingivalis capsular polysaccharide to epithelial cells and defined populations of relevant immune cells. We will characterize 1- dose- and time-dependent binding kinetics, 2- binding specificity, and employ blocking studies to define the attachment of purified P. gingivalis capsular polysaccharide to host cells. 2): To characterize the innate immune response of epithelial cells and relevant leukocyte populations to P. gingivalis capsular polysaccharide. Cytokine secretion, cell adhesion molecule production and neutrophil recruitment will be examined. We will delineate the cytokine, chemokine and cell adhesion molecule repertoire produced by cells challenged as related to stimulation and characterization of PMN transmigration. 3): To define the cellular inflammatory event that occurs in response to Porphyromonas gingivalis capsular polysaccharide in a murine air pouch model. We will characterize the host cellular inflammatory response to purified P. gingivalis capsular polysaccharide and begin to define the innate immune responses that govern leukocyte recruitment.

Grant: 1R01DE014924-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: HAN, YIPING W BS BIOCHEMISTRY
Title: Molecular Mechanism of F. Nucleatum Virulence
Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH
Project Period: 2003/08/01-2007/04/30

DESCRIPTION: *Fusobacterium nucleatum* is a Gram negative, anaerobic bacterium ubiquitous to the oral cavity and often associated with periodontal disease (PD). It is also found in infections and abscesses of other parts of the body. In particular, it is implicated in Preterm birth (PTB). PD is newly recognized as a potential risk factor for PTB. Nevertheless, the mechanism underlying the correlation has not been elucidated. As a result of its association with PD and PTB, *F. nucleatum* is an excellent candidate to investigate the relationship between oral health and PTB. The focus of this study is to investigate *F. nucleatum* interactions with host cells in vitro, as a prelude to future analyses of the pathogenic role of *F. nucleatum* in PTB. A novel adhesin, FadA (*Fusobacterium* adhesin A), has been identified that binds to host cells. In addition, it appears to be involved in the coaggregation between different bacteria, a key characteristic in Dental plaque formation. Our hypothesis is that FadA is an important virulence factor for *F. nucleatum* to adhere to and invade host tissue cells and to induce host innate immune responses. The Specific Aims are: 1) To characterize the FadA adhesin from *F. nucleatum* for its role in tissue cell attachment and invasion. 2) To identify the host receptor for FadA and to characterize its role in *F. nucleatum*-host cell interactions. The innovative aspect of this study is investigation of the molecular mechanism of the virulence of *F. nucleatum*. Our long-term goal is to understand the role of *F. nucleatum* in PD and PTB, and to identify the relationship between oral health and maternal health. Knowledge obtained from this study may enable future development of therapeutic agents to reduce the incidence of PD, PTB, and possibly other common systemic infections.

Grant: 1R01DE015510-01
Program Director: NOKTA, MOSTAFA A
Principal Investigator: WEINBERG, AARON PHD IMMUNOLOGY AND MICROBIOLOGY
Title: Oral Mucosal Beta Defensins and HIV
Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH
Project Period: 2003/07/15-2007/05/31

DESCRIPTION (provided by applicant): The mechanisms of resistance to HIV-1 infection in the human oral cavity are incompletely understood. While salivary components have been implicated in protection, there is growing evidence that human defensins may be playing an important role in prevention of HIV infection. New antiviral, chemotaxis and immunosurveillance properties are being attributed to beta-defensins; small cationic antimicrobial innate response molecules expressed in mucosal epithelium. Inducible beta-defensins are always expressed in normal oral epithelium, a property not shared by other mucosal barriers. Certain oral commensal bacteria may contribute to this induction. We have shown that normal human oral epithelial cells (NHOECs) express the known beta-defensins and that *Fusobacterium nucleatum*, a ubiquitous bacterium of the oral cavity, upregulates human beta-defensin 2 (hBD2), resulting in protection from invasive bacteria. We now demonstrate that HIV-1 X4 and R5 viral phenotypes induce hBD2 mRNA in NHOECs and that hBD2 and -3 inhibit HIV-1 infection by both viral strains, with greater activity against X4 viruses. This proposal intends to test hypotheses emanating from the postulate that oral epithelial cells can be stimulated to produce beta-defensins that protect the host from HIV-1 at the oral mucosal barrier. The objectives of this proposal are (1) to study mechanisms of induction of hBD2 by HIV-1 in NHOECs, (2) to analyze the mechanisms by which hBD2 and -3 inhibit HIV-1 infectivity, and (3) to test the *F. nucleatum* hBD2 induction model for protection against HIV-1. Relative resistance to infection by the X4 phenotype, at mucosal sites, may in part be attributed to beta-defensins. Recent developments in defensin biology, coupled with our new observations, will facilitate rapid exploration of this newly discovered antiviral defense mechanism and may lead to future studies of HIV microbicidal strategies and mucosal barrier protection.

Grant: 1R01DE015512-01
Program Director: NOKTA, MOSTAFA A
Principal Investigator: POPE, MELISSA J PHD MICROBIOLOGY &
IMMUNOLOGY
Title: Dendritic cell defensin and type I IFN responses in HIV
Institution: POPULATION COUNCIL NEW YORK, NY
Project Period: 2003/09/01-2007/06/30

DESCRIPTION (provided by applicant): The dendritic cell (DC) system, comprising the myeloid (MDC) and plasmacytoid (PDC) subsets, orchestrates innate and adaptive immunity to pathogens. DCs are located within the epithelial tissues (Langerhans cells, LCs) and in the underlying lymphoid follicles (PDCs and MDCs) that line the oral cavity. Therefore, DCs are in prime positions to encounter oral pathogens and may play an important role in sustaining a healthy oral mucosa. Increasing evidence highlights how DCs both produce and respond to innate factors such as type I IFNs and defensins that possibly provide important barriers against HIV infection as well as in controlling commensal organisms in the oral cavity. We hypothesize that unlike HIV, organisms like herpes simplex virus (HSV) or *Candida albicans* will trigger strong innate type I IFN and defensin responses in DCs that contribute to the resistance of the oral mucosa to HIV infection as well as controlling HSV and *Candida* infections in healthy people. However, prior exposure to HIV will impede these innate DC responses rendering individuals more susceptible to HSV infection and reactivation as well as candidiasis. Three major questions will be addressed to investigate this. 1. Are defensin responses in LCs induced by HIV and organisms present in the oral cavity? 2. What are the innate type I IFN and defensin responses of PDCs and MDCs to HIV and oral pathogens? 3. Do epithelial cells and keratinocytes influence the innate responses of DCs to HIV and oral pathogens? By investigating these issues we will reveal the innate responses of distinct DC subsets that are found in the tissues of the oral cavity to HIV and related co-pathogens, how these responses are influenced by cell-cell (DCs, epithelial cells, and keratinocytes) contact, and whether prior exposure to one organism alters a DC's response to another. The involvement of specific receptors (toll like receptors, TLRs and C-type lectin receptors, CLRs) will be examined to elucidate their role in capture of a pathogen vs signaling of cellular responses. These extensive in vitro studies will uncover pertinent information about the innate responses of DCs to various infections and how these contribute to a healthy oral environment that is perturbed in HIV infected individuals. This will afford critical insight as to how such responses could be boosted to prevent opportunistic infections and also identify potential targets for the development of strategies to prevent HIV transmission across the oral and other mucosal surfaces.

Grant: 1R03DE014447-01A2
Program Director: MANGAN, DENNIS F.
Principal Investigator: HAN, YIPING W BS BIOCHEMISTRY
Title: Fusobacterium nucleatum in Amniotic Fluid Infection
Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH
Project Period: 2003/01/01-2004/12/31

DESCRIPTION (provided by applicant): Preterm birth (PTB) is the number one cause of infant mortality and morbidity. Amniotic fluid (AF) infections are prevalent in PTB. One hypothesis to explain this is that the organisms originate from the vagina and ascend into the uterus. However, some organisms from AF appear to be of oral origin. One of the most frequently isolated species from AF, *Fusobacterium nucleatum*, is highly prevalent in periododontal plaques and infections. *F. nucleatum* is capable of invading human gingival epithelial and umbilical cord vein endothelial cells. Preliminary studies revealed that haematogenous infection of *F. nucleatum* induced pregnancy complications in mice. Therefore, it is reasonable to speculate that the hematogenous route of transmission may also occur in humans. To test this possibility, we aim to investigate the source of the *F. nucleatum* infection in AF. AF samples will be collected via amniocentesis from 400 patients in preterm labor with intact fetal membranes at a gestational age of < 32 weeks. Vaginal, blood, and subgingival plaque samples from these patients will also be collected. AF infections by all bacteria and by *F. nucleatum*, along with the control urogenital species, *Ureaplasma urealyticum*, will be examined by polymerase chain reactions (PCR) using primers specific for the conserved and hypervariable regions of 16S ribosomal RNA, respectively. Once *F. nucleatum* is identified in AF, the vaginal, blood, and plaque samples of the same patient will be examined by PCR. In addition, since *F. nucleatum* is highly heterogeneous, in order to clearly identify the source of infection, *F. nucleatum* will be isolated and identified to the subspecies level and differentiated by DNA fingerprinting. The results from this pilot study will enable subsequent investigation into the mechanism of infection, identifying virulent strains of AF-associated *F. nucleatum* for diagnostic purposes, and intervention studies aimed at reducing the incidence of preterm birth.

Grant: 1R03DE014937-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: PINA, SOPHIA E BA
Title: Gene Regulation of Fimbriae in *Actinomyces naeslundii*
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR SAN SAN ANTONIO, TX
ANT
Project Period: 2003/09/01-2005/05/31

Actinomyces naeslundii is a resident of the normal oral flora, however, it also has the potential to cause disease, namely root caries and periodontitis (3, 4). Colonization by *A. naeslundii* can begin during infancy; it can adhere to salivary proline rich proteins on a tooth surface via type 1 fimbriae and/or to surrounding *Streptococcus* species such as *S. oralis* via the type 2 fimbriae. In addition, adherence by type 2 fimbriae is associated with lactose sensitive receptors in the host, such as those on mucosal epithelial cells, erythrocytes, and polymorphonuclear leukocytes (55, 57). Since little is known about the fimbriae associated adherence mechanisms in gram-positive bacteria, studies of *A. naeslundii* type 1 and type 2 fimbriae make this organism an ideal model to investigate adherence to host cells as well as aggregation to surrounding bacterial cells in a biofilm. This investigation will examine the effects of environmental signals on the synthesis of type 1 and type 2 fimbriae to test the hypothesis that environmental factors can regulate gene expression of fimbriae. The focus of this proposal will include: Aim 1. Determination of environmental signals that regulate fimbrial biosynthesis Real-time reverse transcriptase PCR will be used to monitor the expression of the type 1 and type 2 fimbrial genes of *A. naeslundii* while growing in different conditions in a continuous culture system. Aim 2. Characterization of the type 2 fimbrial promoter The transcriptional start site for the type 2 fimbrial locus will be mapped by primer extension and S1 nuclease experiments. The type 2 fimbrial promoter regions will then be cloned into a reporter gene vector for use, in future grant periods, in promoter mutation studies.

Grant: 1R03DE015241-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: LEWIS, JANINA P
Title: Prevotella intermedia: Iron and Virulence
Institution: VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA
Project Period: 2003/07/01-2005/04/30

DESCRIPTION (provided by applicant): Prevotella intermedia is associated with both oral (periodontal disease and endodontic infections) and extraoral infections. Despite this organism's association with disease little is known about the genetic or biochemical basis of its virulence. An essential step in establishment of an infection is the ability of a pathogen to multiply within its host. This property is greatly influenced by availability of nutrients such as iron. Hemin has been shown to be an indispensable nutrient for these bacteria. It serves as a source of iron and protoporphyrin IX. Thus, we will start our study of the virulence features of this bacterium by investigating the molecular mechanisms of hemin acquisition. First, we shall identify the genes involved in the pigmentation locus. In oral gram-negative bacteria, black pigmentation results from accumulation of hemin on the cell surface. We hypothesize that there are several genes coding for the proteins participating in the integration of hemin. Furthermore, we hypothesize that the genes involved in hemin/iron transport are coordinately regulated by iron. Proteomics is the best approach to rapidly identify large numbers of differentially regulated gene products. Using this approach we intend to identify proteins affected by iron depletion. The results of our study will help define iron uptake mechanisms that may serve as targets in the future design of antibacterial agents for P. intermedia. Since P. intermedia is resistant to many antibiotics alternative methods of preventive measures are needed for this bacterium.

Grant: 1R03DE015441-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: KOO, HYUN DDS
Title: Effects of Natural Agents with Fluoride on Caries
Institution: UNIVERSITY OF ROCHESTER ROCHESTER, NY
Project Period: 2003/08/01-2005/05/31

DESCRIPTION (provided by applicant): Apigenin and tt-farnesol, were identified as potentially novel natural anti-caries agents (Koo et al., 2002b; c). Both agents exhibited cariostatic properties in rats without significant effects on the animals' oral flora. Apigenin is a potent inhibitor of glucosyltransferases (GTFs), both in solution and on a surface (60-95% inhibition at 1.33mM), and without effect on bacterial growth, tt-Farnesol, in contrast, affects the growth rate and metabolism of mutans streptococci biofilms by disrupting the membrane function. The aim of the proposed study is to evaluate the effects of the combination of apigenin, tt-farnesol and fluoride on the formation and composition of mutans streptococci biofilms in vitro, and on caries development in rats. Fluoride is a clinically proven anti-caries agent; its main effect is to interfere physicochemically with caries development. However, fluoride has antibacterial activity, and in addition, may interfere with GTF production (Bowen and Hewitt, 1974). Our hypothesis is that the association of the natural agents may enhance the anti-caries effect of fluoride by synergistically diminishing the virulence factors of mutans streptococci involved in the pathogenesis of dental caries. The rationale for this study is that the combination of the therapeutic agents will reduce the formation and virulence of cariogenic biofilms by (a) inhibiting the synthesis of glucans, e.g., inhibition of the activity and production of GTFs; (b) reducing the acid tolerance, e.g., inhibition of F-ATPase; (c) reducing the acidogenicity, e.g., enhancing the access of fluoride into cells by increasing the bacterial membrane permeability. We will test our hypothesis using a series of experiments as follows: I) In vitro- By determining the effects of the combination of agents on (1) formation, (2) viability, (3) pH, and (4) polysaccharide and inorganic composition of mutans streptococci biofilms. Streptococcus mutans biofilms formed on hydroxyapatite disks will be used in this part of the investigation. The polysaccharide composition will be determined by a series of colorimetric assays, liquid scintillation counting and gas chromatography/mass spectrometry. The concentrations of fluoride will be determined using a fluoride-selective electrode, calcium will be analyzed by atomic absorption spectrophotometry and phosphorus will be determined colorimetrically. The pH of the biofilms will be measured using a Beetrode pH electrode. II) In vivo - By evaluating the ability of combination(s) of agents (selected from in vitro analyses) to reduce dental caries, and to affect the pH and the composition of plaque using our rat model. The outcome of this study may lead to new and effective therapeutic combinations interventions to prevent dental caries, and possibly other plaque related diseases by using novel compounds in association with a well-known anti-caries agent.

Grant: 1R03DE015501-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: WEN, ZEZHANG PHD
Title: LuxS-Mediated Quorum Sensing in Streptococcus mutans
Institution: UNIVERSITY OF FLORIDA GAINESVILLE, FL
Project Period: 2003/06/15-2005/05/31

DESCRIPTION (provided by applicant): Streptococcus mutans is recognized as the principal etiological agent of dental caries, the most prevalent infectious disease of humans. The ability to metabolize carbohydrates and generate acids, to survive acidic pH and other adverse conditions, and to adhere to and form tenacious biofilms on the tooth surface are believed to be critically associated with the cariogenicity of this human pathogen. Known for its high degree of acid tolerance (aciduricity) and its high capacity to produce acid (acidogenicity), S. mutans lives primarily on the tooth surface at high cell-density in a high diversity ecosystem better known as dental plaque, the structure and composition of which is known to be largely influenced by such factors as the source and availability of nutrients, the pH in the oral cavity and by the ability of the biofilm organisms to adapt to the fluctuations in environmental conditions. Quorum sensing is a cell density--dependent regulatory mechanism that is known to be involved in regulation of a variety of physiologic processes and virulence in both Gram (+) and Gram (-) bacteria. We have recently generated evidence that the S. mutans possesses a gene encoding a functional homologue of the new family of autoinducer synthases (LuxS) that are responsible for production of autoinducers of the quorum sensing system 2, AI-2. This study is designed to yield novel information concerning LuxS-mediated quorum sensing and virulence regulation in S. mutans, which will contribute to our understanding of the pathogenesis of this microorganism and the ecology of the oral flora. The Specific Aims of this proposed study are: 1) to investigate the role of luxS in acid tolerance by S. mutans. By using functional assays, reporter gene fusions, Northern hybridization, and proteomics, we will investigate acid tolerance and its regulation by luxS, and identify novel factors (proteins) that are involved in luxS-regulated acid tolerance responses. 2). To use confocal laser scanning microscopy (CLSM) and mixed, known-species consortia to determine the impact of luxS of S. mutans on bacterial adherence by S. mutans and the inter- and intra-generic interactions between S. mutans and other oral bacteria in terms of biofilm initiation development and structure.

Grant: 1R15DE014854-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: SPATAFORA, GRACE A PHD BIOLOGY
Title: Regulation of Streptococcus mutans Virulence by covR/S
Institution: MIDDLEBURY COLLEGE MIDDLEBURY, VT
Project Period: 2003/01/01-2004/12/31

DESCRIPTION (provided by applicant): Two component signal transduction systems are widely used by pathogenic bacteria to regulate the expression of their virulence factors in response to changing environmental cues during the infectious process. Streptococcus mutans is exposed to the transient environments of the human oral cavity where it is the primary etiologic agent of Dental caries. Specifically, changes in pH, oxygen content, and nutrient availability are likely to necessitate a rapid bacterial response to promote S. mutans-induced cariogenesis. We identified a gene pair in S. mutans that is homologous to the covR/S two-component signal transduction system in the group A streptococci (GAS). CovR/S is known to regulate the expression of multiple genes associated with GAS virulence, including basal which is necessary for capsule production and ska, which encodes a plasminogen activator. Work conducted in5laboratory implicates the S. mutans covR/S homologs in the regulation of fructosyltransferases (ftf) that mediate the sucrose-dependent production of fructans necessary for bacterial adherence to the tooth pellicle. Since S. mutans produces a multitude of factors that promote its survival and persistence in the human host, we posit that CovR/S may function as a global regulator of S. mutans genes whose products promote disease in the oral cavity. The major goal of this research application is to define a putative role for CovR/S in S. mutans virulence control. The Specific Aims include: 1. Characterization of a S. mutans covR- mutant recently constructed in5laboratory, and analysis of its cariogenic potential in germfree rats; 2. Identification of the S. mutans gene(s) that are subject to CovR/S control in environments that approximate the oral cavity using differential display polymerase chain reaction (ddPCR) and 2D proteomic approaches; 3. construction of S. mutans knockout mutants and reporter gene fusions to functionally characterize CovR/S-regulated genes and analyze their expression. Taken collectively, these studies will elucidate S. mutans mechanism(s) of virulence gene control, and so enable prevention and/or intervention in the pathogenic process that leads to the development of Dental caries.

Grant: 1R21DE014583-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: LEVINE, MARTIN
Title: Lysine decarboxylase in periodontitis patients
Institution: UNIVERSITY OF OKLAHOMA HLTH SCIENCES CTR OKLAHOMA CITY, OK
Project Period: 2003/07/01-2005/05/31

DESCRIPTION (provided by applicant): In chronic (formerly adult) periodontitis, bacteria destroy the dento-gingival junction, especially its Dentally attached (DAT) cells, keratinocytes that are activated by persistent trauma from mastication and oral hygiene. These rapidly dividing cells form the internal basement lamina of junctional epithelium (JE) and maintain the epithelial attachment. The DAT cell coronal extremity grows on interstitial fluid that transudes through the JE to the base of a gingival sulcus, near the site of infecting bacteria. We posit that lysine decarboxylase (LDC), a bacterial enzyme in the sulcus, depletes the transudate of lysine, starving the DAT cells. A small amount of LDC causes a cascade of events predisposing to loss of Dental attachment and colonization of the sulci by well-known periodontopathogens. Oral hygiene controls this colonization, but some adults are refractory and discriminated by increased Capnocytophaga spp. and other LDC producers. The activity of LDC from the bacteria in gingival sulci may be critical for determining whether chronic periodontitis can be controlled by current (oral hygiene-based) therapy. The Aims of this study are to: 1) develop new assays for measuring the amount of active LDC in the gingival microbiota (plaque); and 2) use these assays for measuring active enzyme in the sulci from refractory and successfully treated patients. LDC activity will be determined by two methods. The first will determine enzyme activity (cadaverine synthesis) in the presence of a saturating amount of substrate (lysine) in extracts of whole-mouth plaque from refractory and successfully treated patients. The second will use H-a-difluoromethyl DL-lysine (DFML), a suicide inhibitor that forms an adduct at the catalytic center of LDC. DFML is not commercially available and it will be synthesized unlabeled and as a radioactive derivative for this project. Radiolabeled adduct formation should be inhibited by an excess of unlabeled L-DFML or lysine and the amount of radioactivity on blots will indicate the amount of active enzyme after incubation with plaque extracts. The application predicts that there will be more enzyme activity in refractory than in successfully treated patient plaque. The results will indicate the relationship of LDC activity in the gingival sulcular microbiota to therapeutic outcome, and whether LDC inhibitors such as DFML might have utility as a new, alternative pharmacotherapeutic for preventing and controlling chronic periodontitis.

Grant: 1R21DE014587-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: QUARANTA, VITO
Title: P.Gingivalis and Oral Keratinocyte-ECM Interactions
Institution: VANDERBILT UNIVERSITY NASHVILLE, TN
Project Period: 2003/08/01-2005/05/31

DESCRIPTION: The goal of this application is testing the novel hypothesis that the periodontal pathogen *Porphyromonas gingivalis* (P.g.) interferes with the interactions between oral keratinocytes (OK) and extracellular matrix (ECM), and may cause loss of epithelial integrity. P.g. is a well-known oral pathogen with several distinct virulence factors, which may contribute to P.g. pathogenicity. We intend to focus on those factors that directly or indirectly affect OK/ECM interactions. Our preliminary data show that P.g. alters OK cell morphology, adhesion, migration and survival, and induces proteolysis of OK proteins. Our project is articulated into 3 Aims, with the purpose of investigating the molecular mechanisms, both cellular and bacterial, which may underlie these P.g. effects on ECM related functions in OK. In Aim 1, we will analyze the specificity of P.g. interference towards ECM macromolecules that are characteristically found either in normal (e.g. laminin-5 and collagen) or in chronically diseased (e.g. fibronectin and vitronectin) periodontal tissue. To this end, adhesion, migration and survival assays on specific ECMs will be performed with continuous OK cell lines in the presence of P.g. Furthermore, we will compare cellular effects of P.g. with those possibly induced by other oral bacteria, both pathogenic and not. In Aim 2, we will determine whether P.g. alters ECM related functions in OK by interfering with integrin expression and activation state, cytoskeletal organization, and/or modification of integrin-proximal and distal transduction pathways, focusing on the effect of P.g. gingipains. In Aim 3, we will test specific P.g. and other bacterial factors such as proteinases and fimbriae for their possible effects on OK/ECM interactions, by comparing P.g. wild-type versus well-defined virulence mutant strains, using as a guide data from Aim 1 and 2. Results from these approaches will hopefully provide new and exciting molecular clues as to the mechanisms whereby P.g. may initiate and sustain periodontal disease. Such clues may be useful for devising new modalities of treatment and prevention.

Grant: 1R21DE014926-01
Program Director: BHARGAVA, SANGEETA
Principal Investigator: DAO, MY LIEN PHD
Title: Efficacy of DNA versus Protein Vaccine
Institution: UNIVERSITY OF SOUTH FLORIDA TAMPA, FL
Project Period: 2003/07/15-2005/05/31

DESCRIPTION (provided by applicant): Dental caries is an infectious disease caused by *Streptococcus mutans*. Despite fluoridation and improved Dental hygiene in industrialized countries, worldwide Dental caries is still a significant public health problem affecting 50-90 percent of the population (World Health Organization). In the United States of America, 59 percent of first graders have at least one cavity, one third of the adult population does not see a Dentist annually, and 24 percent of the elderly experience caries-associated tooth loss (Oral Health America). Animal studies demonstrated that this disease was preventable by immunization with *S. mutans* antigens. Gene cloning technology was applied to produce recombinant bacteria, protein and peptide vaccines for active immunization, and antibody for passive immunization. Various levels of protection were achieved with these vaccines. Considering that the population at risk is mostly from low socioeconomic groups (Surgeon General's report, U.S. DHHS, 2000), it is desirable to find means to lower the costs of vaccine production for mass immunization. One possibility is to directly immunize with a plasmid DNA (cDNA) containing the gene(s) of interest and obtain expression of the target protein(s) in the host. Thus, expensive protein isolation from recombinant clones is avoided. The Long Term Goal of the current study is to prepare an efficacious, safe and economical Dental Caries vaccine. The Specific Aim for the proposed period is to explore the prospect of a DNA vaccine against *S. mutans* with special emphasis on comparing the efficacy, duration and costs with those of corresponding Protein Vaccine. As models, the *S. mutans* antigen A (AgA), a recognized candidate vaccine antigen, and its precursor the wall-associated protein A (WapA), a factor involved in colonization and buildup of Dental plaque, will be used. The work proposed is supported by the availability of the recombinant clones needed for the production of WapA and AgA protein vaccines, and wapA-pDNA and agA-pDNA vaccines, and by the expression of WapA and AgA in mammalian cells transfected with these pDNA constructs. The results obtained will determine the feasibility and cost-effectiveness of genetic immunization against Dental caries, and the work performed will serve as a model for vaccine research against other infectious agents invading the body through mucosal surfaces.

Grant: 1R21DE015127-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: DAO, MY L PHD
Title: Cloning Analysis of *S. mutans* Putative Collagenase
Institution: UNIVERSITY OF SOUTH FLORIDA TAMPA, FL
Project Period: 2003/08/01-2005/05/31

DESCRIPTION: Dental root decay is prevalent among older individuals as their gum recesses exposing Dental root surface to attack by cariogenic bacteria. In a study involving 449 subjects of an age range of 79-101 years, 96% had coronal decay experiences, and 64% had root caries experience with 23% of the group having untreated root caries (ADA News Releases, 2000). *Streptococcus mutans*, an etiologic agent in the development of coronal caries, has also been implicated in Dental root decay; data in support of this implication include the finding of *S. mutans* in Dental root section, its ability to bind collagen, and to degrade FALGPA, a known synthetic peptide substrate for collagenase. Bacterial collagenases are considered as virulence factors as they facilitate the invasion and destruction of host tissues by the pathogens. It is not yet known whether *S. mutans* produces a true collagenase enzyme. Considering the increase in incidence of Dental root caries as the population lives longer, the long-term goal of the current study is to develop effective and safe methods to control this disease, and improve the nutrition and quality of life of the population at risk. In order to determine whether the collagenolytic enzyme in *S. mutans* is a good candidate antigen for vaccine development, the Specific Aim of the current research is to learn more about the *S. mutans* enzyme in order to explore this avenue. A putative *S. mutans* collagenase gene has been obtained previously, and sequence analysis showed a high homology with the 35-kDa collagenase of various clinical isolates of *Porphyromonas gingivalis*, a bacterium causing periodontitis. The plan is to clone the 1.2 kbp putative collagenase coding sequence into an expression plasmid under the control of a strong promoter in order to obtain the corresponding protein, which in turn will be isolated and characterized by biochemical methods. Antibody will be prepared against the *S. mutans* enzyme and tested for the ability to block collagen binding and/or collagen degrading properties. The data obtained will be compared with other known bacterial collagenases. This information is essential in determining future directions for research on the role of *S. mutans* in Dental root caries, and other diseases that may involve collagen binding and collagen degrading activity such as periodontitis and endocarditis.

Grant: 1R21DE015360-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: QUIRYNEN, MARC PHD
Title: Guided Pocket Recolonization (GPR) in Periodontitis
Institution: CATHOLIC UNIVERSITY OF LOUVAIN BELGIUM,
Project Period: 2003/04/04-2005/03/31

DESCRIPTION (provided by applicant): Periodontitis is an infectious disease resulting in an often-painless destruction of tooth supporting tissues (the periodontium) and enhances the risk for various systemic diseases including atherosclerosis, coronary heart disease, stroke and infants with low birth weight. Active periodontitis occurs in a susceptible host and in the presence of pathogenic species in combination with low concentrations of so-called "beneficial bacteria". The success of periodontal therapy primarily depends upon dealing with the negative environmental/behavioral factors and the reduction/elimination of periodontopathogens in combination with the re-establishment of a more suitable environment (less anaerobic) for a beneficial microbiota. Even after therapy, the presence of pathogenic species in subclinical levels is often encountered so that there remains a continuous threat for further periodontal destruction and disease. Therefore, this project aims to improve treatment outcome by guiding the periodontal pocket recolonization after periodontal therapy by local administration of beneficial bacteria (probiotics). An optimal mixture of known beneficial periodontal bacteria will be determined in vitro by investigating the adhesion of these bacteria to dentine- and enamel surfaces and epithelial cells in relation to their probiotic effect using flow cell microscopy and fluorescence microscopy. The in vivo effects of the therapy will be evaluated using a split mouth study design in a beagle dog model for periodontitis. After lesion induction and establishment of severe periodontitis in 8 beagle dogs (20 lesions/dog), 8 lesions do not receive treatment (reservoir for pathogenic recolonization of the treated lesions), 4 lesions receive mechanical debridement (classic strategy), 4 lesions receive mechanical debridement followed by a single subgingival application of the probiotic mixture (experimental treatment 1) and 4 lesions receive mechanical debridement followed by a repeated subgingival application (week 1, 2 and 4) of the probiotic mixture (experimental treatment 2) in each dog. Plaque samples will be obtained from all pockets after 2, 4, 6, 8, and 12 weeks and evaluated via standard culture techniques and checkerboard DNA-DNA hybridization. Clinical data will be collected after 2, 4, 6, 8 and 12 weeks. The results should indicate the possible role for commensal organisms in periodontal disease and subgingival biofilm formation.

Grant: 1R21DE015361-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: REYNOLDS, ERIC C PHD
Title: Impact of Oral bacterial interactions on periodontitis
Institution: UNIVERSITY OF MELBOURNE MELBOURNE,
Project Period: 2003/06/01-2005/05/31

DESCRIPTION (provided by applicant): Periodontal diseases are bacterial-associated inflammatory diseases of the supporting tissues of the teeth, and one of the more aggressive forms of chronic periodontitis is characterized by the destruction of the tooth's supporting structures. Chronic periodontitis has a large economic burden and is a major public health problem. Three bacterial species (*Porphyromonas gingivalis*, *Treponema denticola*, *Bacteroides forsythus*) have been shown to be closely associated with chronic periodontitis in humans, and a commensal oral bacterium *Fusobacterium nucleatum* has been implicated in aiding the colonization of the periodontopathic bacteria. *P. gingivalis*, *T. denticola*, *B. forsythus* and *F. nucleatum* exist as part of a high density mixed species (polymicrobial) biofilm in the gingival crevice of humans. Recent evidence using rodent abdominal lesion models has indicated that co-infection of *B. forsythus* or *T. denticola* with *P. gingivalis* enhances lesion development. This synergistic interaction that enhances lesion severity, and potentially virulence of the bacteria, has not been studied in an animal periodontitis model. Further, outer membrane proteins that are altered during this interaction also have not been studied. The broad objective of this application is to determine the impact of interactions between *P. gingivalis*, *T. denticola*, *B. forsythus* and *F. nucleatum* on the level of bone loss and host antibody response in the murine periodontitis model and to characterize the major antigenic outer membrane proteins of these bacteria using 2D PAGE and mass spectrometric methods. Using these techniques, we have already identified over thirty *P. gingivalis* outer membrane proteins. We have also established a murine periodontitis model based on oral infection of *P. gingivalis*. These methodologies will be applied to determine the impact of bacterial interactions on the expression of major antigenic outer membrane proteins and on colonization, bone loss and host immune response. The significance of this work is that it will increase our knowledge of the role of bacterial interaction on polymicrobial virulence in the murine periodontitis model and may have a broader significance in the study of human mucosal pathogens. The specific aims of the application are: 1) To determine the extent of alveolar bone loss and level of bacterial colonization in the murine periodontitis model when co-inocula of *P. gingivalis*, *T. denticola*, *B. forsythus* and/or *F. nucleatum* grown as monocultures are used to orally infect mice compared with mono-inoculation of each species. 2) To determine the extent of alveolar bone loss and bacterial colonization in the murine periodontitis model when polymicrobial cultures of *P. gingivalis*, *T. denticola*, *B. forsythus* and/or *F. nucleatum* are used to orally infect mice compared with mono- and co-inocula of the bacteria grown as monocultures as in (1) above. 3) To determine the host antibody isotype response to each of the bacteria in the murine periodontitis model when co-inocula or polymicrobial cultures of *P. gingivalis*, *T. denticola*, *B. forsythus* and/or *F. nucleatum* are used to orally infect mice. 4) To identify the major outer membrane and antigenic proteins of *P. gingivalis*, *T. denticola*, *B. forsythus* and *F. nucleatum* grown in monoculture and as polymicrobial cultures using 2D-gel electrophoresis, mass spectrometry and Western blotting.

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Grant: 1R21DE015499-01
Program Director: NOKTA, MOSTAFA A
Principal Investigator: TAO, LIN PHD MEDICAL
MICROBIOLOGY & IMMUN
Title: Blocking Milk-Borne HIV by Commensal Bacteria
Institution: UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL
Project Period: 2003/07/10-2005/04/30

DESCRIPTION (provided by applicant): This application is in response to the NIH RFA (DE-03-002) entitled "Oral Mucosal Innate Immune Factors in the Inhibition of HIV and Opportunistic Infections." Specifically, we will study a novel factor of the oral innate immunity--mannose-binding lectin (MBL)--of commensal bacteria. The rationale is that the human MBL binds and neutralizes HIV, but it exists only in serum, not on mucosal surfaces where most HIV infections occur. We think that the lack of host MBL on mucosal surfaces can be compensated, at least in part, by MBL-like lectins of commensal bacteria. Our preliminary studies showed that some lactobacilli that colonize the oral cavity produce moderate levels of MBL-like lectin. Like the human MBL, microbial MBL-like lectins also bind HIV because the HIV receptor, gp120, is rich in mannose-residues. Lactobacilli, which naturally grow in milk (lacto=milk), are earliest colonizers of the oral and intestinal mucosa in milk-fed infants and are a beginning component of the infant's innate immunity against infectious diseases, such as diarrhea. However, the affinity of microbial MBL-like lectins are not high enough to block HIV infections. We hypothesize that oral lactobacilli that produce abundant MBL-like lectins with a high affinity against HIV can be isolated from spontaneously occurred and/or induced mutants. We will obtain these super-MBL lactobacilli by pursuing two specific aims: 1) Obtain super-MBL Lactobacillus strains by screening spontaneously occurred and/or induced mutants. 2) Test the ability of super-MBL lactobacilli to block HIV viruses in vitro Upon completion of this study, we will have obtained a collection of super-MBL oral lactobacilli that bind and neutralize HIV with high efficiency. These bacteria will be used for further studies on their binding characteristics, safety, in vivo efficacy and colonization in animals and ultimately in human volunteers. The long-term goal of this project is to select food-grade commensal bacteria that naturally trap and kill HIV viruses and to develop a bioprophylactic therapy to safely block the vertical transmission of HIV via breastfeeding.

Grant: 1R21DE015508-01
Program Director: NOKTA, MOSTAFA A
Principal Investigator: GARZINO-DEMO, ALFREDO PHD
Title: HIV suppression by Beta-defensins
Institution: UNIVERSITY OF MD BIOTECHNOLOGY BALTIMORE, MD
INSTITUTE
Project Period: 2003/07/15-2005/05/31

DESCRIPTION (provided by applicant): beta-Defensins are small (3-5Kd in size) secreted proteins that are components of innate immunity; some are constitutively expressed, such as human beta-defensin (HBD)-1, while others, like hBD2 and -3, are inducible by cytokines or other immune response stimuli. beta-defensins are secreted preeminently by epithelial cells, and by neutrophil cells, although their secretion has been observed also in T and NK cells, and initially they were described as antimicrobial proteins; recent research has indicated that they also act as chemoattractants. Their expression is elevated in the epithelia of the mouth, tongue, digestive apparatus, and also in airways, mammary gland, liver and other organs. In particular, beta-defensins are present in saliva, and the concentration of these proteins can be very high in the oral cavity, with measured local concentration as high as 100mu g/ml, in a 100mu m-thick layer in the tongue. Therefore, it appears that beta-defensins, as important component of the innate immunity, control the occurrence of infections in the oral cavity. The antimicrobial activity of beta-defensins is due to their ability to permeabilize bacterial membranes. Taken together, this information indicates that beta-defensins could provide a form of innate immunity against oral HIV infection that might be exploited for anti-HIV prophylaxis. In agreement, our preliminary data show that select beta-defensins, especially hBD2, inhibit R5 HIV infection in a dose dependent manner, at doses that are compatible with or below those measured in the oral cavity. In addition, our studies show that hBD2 treatment directly on the virus lowers HIV infection. This antiviral activity is reminiscent of the recently reported HIV suppressive properties of alpha defensins. However, only beta-defensins are naturally present in the oral cavity at HIV-suppressive concentrations. Therefore, our central hypothesis is that beta-defensins mediate and antiretroviral mechanism, based on inhibition of viral entry, in the oral cavity that is capable of preventing oral HIV transmission. Accordingly, we propose to a) characterize which HIV- 1 phenotypes are suppressed by hBD2. These experiments will define the broad suppressive effects of hBD2 and whether its mechanism of suppression functions in vivo b) determine the effects of hBD2 on events in the HIV-1 life cycle. In this aim we will examine and compare the effects of hBD2 on HIV entry/fusion and on intracellular steps of viral replication. The elucidation of the event(s) in HIV infection and expression that are affected by hBD2 will allow for a thorough study of its mechanism of action and for structure-function studies, thus proving basis for novel antiviral therapeutic and preventive strategies. Finally, these studies will constitute a contribution towards the understanding of the role of innate immunity in the oral cavity.

Grant: 2P01DK035108-19
Program Director: HAMILTON, FRANK A.
Principal Investigator: KAGNOFF, MARTIN F
Title: INTESTINAL IMMUNE SYSTEM IN HOST-ENVIRONMENT INTERACTION
Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA
Project Period: 1998/11/01-2008/03/31

The central theme of the Program Project is focused on defining the mechanisms that govern host inflammatory and immune responses at mucosal surfaces in the gastrointestinal tract. The four interrelated projects use microbial pathogens, microbial products, and environmental stress injuries as probes of host intestinal mucosal defense. The program applies state-of-the-art approaches and draws on strengths inherent in human and murine in vitro and in vivo model systems to explore key signaling pathways in host intestinal epithelial cells and macrophages that are required for innate immunity and cell survival. A major focus is placed on defining mechanisms by which bacteria, bacterial products, and mucosal injury signal intestinal epithelial cell and macrophage responses, and mechanisms that are important for the host response to infections with disease-causing noninvasive, minimally invasive and invasive pathogens. The Program brings together experienced investigators from the Departments of Medicine, Pharmacology and Pathology with significant expertise in immunology, molecular biology, biochemistry, and microbiology. Research Unit 1 investigates the functional role and importance of the transcription factor NF-kappaB in intestinal epithelial cells in vivo in regulating intestinal mucosal innate immune responses to microbial pathogens and in preventing epithelial cell apoptosis, as well as the role epithelial NF-kappaB plays in an in vitro model of epithelial cell wound healing. Research Unit 2 examines intestinal mucosal responses and mucosal defense mechanisms that govern the host's interaction with *G. lamblia* and *C. parvum*. Research Unit 3 examines epithelial cell signaling mechanisms that are activated by bacterial DNA and the mechanisms by which bacterial DNA can modulate intestinal mucosal innate immunity and mucosal inflammatory responses in vivo. Research Unit 4 examines the role of the transcription factor NF-kappaB in a model of intestinal ischemia reperfusion injury and the mechanisms by which NF-kappaB, together with p38 MAP kinase, governs macrophage death and survival in response to microbial products. The research projects are supported by four Cores: a Cell Culture and Assay Core, a Mouse Model Core, a Histopathology Core, and an Administrative Core.

Grant: 2P01DK053369-06A1
Program Director: NYBERG, LEROY M.
Principal Investigator: STAMM, WALTER E MD INTERNAL
MED:INFECTIOUS DISEASE
Title: PATHOGENIC MECHANISMS IN URINARY TRACT INFECTION
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 1998/02/24-2008/06/30

An estimated 150 million UTIs occur annually on a global basis, accounting for direct health care costs exceeding 6 billion dollars and making UTIs a very significant public health burden. This program project proposes studies that will improve our understanding of the epidemiology, microbial ecology, molecular pathogenesis and prevention of UTIs. The objectives of the project will be met through the development of a multidisciplinary research team that will collaborate to carry out four interrelated projects. Project 1 will undertake a double-blind placebo-controlled trial to determine whether a *Lactobacillus crispatis* vaginal probiotic will reduce the incidence of recurrent UTIs in women. The trial will also study adherence of the probiotic strain to vaginal epithelial cells from the subjects and will thus provide insight into the host and microbial mechanisms involved. Project 2 will address the hypothesis that cell surface glycosphingolipids in the bladder and vaginal epithelium are structurally organized into pleotrophic plasma membrane assemblies called caveolae and that caveolae participate in the initial epithelial response to attachment and uptake of uropathogenic *e. coli*. Project 3 will employ two novel technologies that were developed during the previous funding period, namely high density microarrays and whole genome mutation scanning to characterize on a genomic level the molecular adaptation that uropathogenic *E. coli* strains undergo in the course of recurrent UTIs and in shifting from an asymptomatic to symptomatic infection. The project will also define how such adaptive evolution affects the ability of uropathogens to bind and invade epithelial cells, induce inflammation and resist phagocytosis. In Project 4, the association of host susceptibility to recurrent cystitis or pyelonephritis with known or novel mutations in specifically selected candidate host genes will be examined. The candidate genes to be studied are toll-like receptors (TLR2, TLR4, and TLR6), chemokine receptors (CXCR1 and CXCR2), and interferon γ receptors (IFN- γ R1 and IFN- γ R2). A laboratory core will provide microbiological studies, primary bladder and vaginal epithelial cells, characterization of urovirulence genes, bacterial adherence assays and other resources to the projects. An Administrative/Biostatistical Core will coordinate the overall project and provide biostatistical expertise to all investigators. The proposed studies will result in an improved understanding of pathogenetic mechanisms in UTIs and will result in new approaches to prevention of UTIs.

Grant: 2R01DK015681-31A1
Program Director: HAMILTON, FRANK A.
Principal Investigator: HAGEN, SUSAN J PHD
Title: GI Mucosal Barrier in Health and Surgical Disease
Institution: BETH ISRAEL DEACONESS MEDICAL BOSTON, MA
CENTER
Project Period: 1977/01/01-2008/05/31

DESCRIPTION (provided by applicant): The overall aim of the proposed work is to understand how *Helicobacter pylori* (HP) infection causes mucosal damage in the stomach, with special emphasis on the fundic region of the stomach. The investigators plan to study how HP infection impairs tight junction integrity and wound repair after injury (restitution) to result in increased mucosal permeability. HP-induced mucosal damage of the stomach is important because it is a major initiating factor in the pathogenesis of HP disease, including inflammation, atrophy of fundic epithelial cells including parietal and chief cells, metaplasia, and progression to gastric cancer. Because HP infection has far-reaching implications in terms of benign and malignant disease, mechanisms that underlie the initiation of mucosal damage, repair after damage, and protection against damage are important and timely. Three hypotheses will be tested. 1) HP infection increases mucosal permeability because either HP or inflammatory cells decrease tight junction integrity. This hypothesis will be tested by investigating how HP sonicates or cytokines secreted during a type 1 T-helper (Th1) response affect the phosphorylation and membrane association of proteins associated with the tight junction. 2) HP infection increases mucosal permeability because ammonia, a cytotoxin produced during HP infection, inhibits restitution. This hypothesis will be tested by investigating the effects of ammonia on activity of the H⁺/lactate transporter, MCT1, and on basolateral K⁺-channel activity. 3) L-glutamine (Gln) supplementation decreases mucosal permeability by inhibiting the Th1 response and cytokine-induced decreases in tight junction integrity that occur during HP infection. This hypothesis will be tested by determining whether L-Gln supplementation inhibits the Th1 cytokine response to preserve tight junction integrity in a mouse model of disease. The proposed investigations follow a logical sequence from previous studies in this laboratory, which have provided a structural framework for our knowledge of mucosal injury, protection against injury, and rapid epithelial repair (restitution) as they relate to maintenance of the gastric mucosal barrier in health and surgical disease.

Grant: 2R01DK046461-10
Program Director: HAMILTON, FRANK A.
Principal Investigator: CZINN, STEVEN J PHD
Title: Mucosal Immunology of Helicobacter Induced Gastritis
Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH
Project Period: 1993/12/15-2006/11/30

Helicobacter pylori (*H. pylori*) is a major cause of chronic active gastritis, primary peptic ulcer disease and is strongly linked to gastric cancer. Individuals infected with *H. pylori* mount immune and inflammatory response which fail to clear the infection and may contribute to disease. Despite the inability of the host to clear this infection, we have shown that mice can be protected from *H. pylori* infection by vaccination. Based on studies performed in the previous funding period, we have demonstrated that CD4 positive T cells, but not antibodies or CD8 positive T cells, are required for protection from *H. pylori*. Using semi-quantitative RT-PCR, we measured gastric cytokine expression in protected and unprotected mice to determine which cytokines were associated with protection. Based on these studies, the elimination of *H. pylori* in an immunized host does not seem to follow any of the existing paradigms for clearance of or protection from a mucosal pathogen. Interestingly, IL-12 was required for protection but many of the typical effector molecules of IL-12 were not required (iNOS, IFN- γ and TNF α). Presently, the best correlate with protective immunity seems to be gastric inflammation. We also observed that inflammation associated with protective immunity was transient and declined to background levels when the infection was eliminated. Therefore, we hypothesize that gastric inflammation not only can lead to serious clinical consequences such as the development of gastric cancer and peptic ulcer disease (if it is chronic), but a transient inflammation may also be required for protection following immunization. In an extension of our previous work, we will focus on identifying the elements of the transient host inflammatory response that mediate protection from *H. pylori* infection. In a second related aim, we will focus on identifying the genes that regulates the severe chronic gastric inflammation associated with persistent *Helicobacter* infection that ultimately results in a subset of infected individuals developing gastric cancer and peptic ulcer disease. Thus, studying the inflammation associated with *H. pylori* infection--both the beneficial aspects which may be associated with vaccination and the bad aspects which are associated with disease--is the common theme of this application.

Grant: 2R01DK051677-06
Program Director: HAMILTON, FRANK A.
Principal Investigator: ERNST, PETER B DVM IMMUNOLOGY
(MICROBIOLOGY)
Title: Cytokine Responses to H. pylori Infection, Immunization
Institution: UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA
CHARLOTTESVILLE
Project Period: 1997/09/01-2007/05/31

DESCRIPTION (provided by applicant): Gastric immune responses interact with bacterial virulence factors to promote the pathogenesis of diseases associated with H. pylori infection. While helper T (Th) cells promote inflammation in response to H. pylori in both humans and mice, they are also essential for immunity in animal models. Effective immunity can be induced by vaccination although antibody responses are not necessary. Since H. pylori is a noninvasive pathogen, the "protective" Th cells are believed to modify epithelial cell gene expression such that the niche favoring colonization is disrupted. Th1 cells can augment expression of receptors that bind H. pylori and favor the epithelial damage that ensues. As such, they act as a "pathogenic" T cell. Either endogenous or exogenous ROS can regulate the expression of genes associated with Th1 cells. In contrast, Th2 cells, through the production of IL-4, IL-5, IL-10, IL-13, IL-25 and TGF-Beta can antagonize the effects of Th1 cells. Tr1 cells, a recently described subset of Th cells, resemble Th2 cells by producing IL-5 and -10 but also produce IFN-gamma similar to Th1 cells. Importantly, Tr1 cells are present in the digestive tract and attenuate the host response to luminal antigen including the induction of colitis in animal models. Cytokines associated with Tr1 cells prevent the generation of ROS, the expression of genes associated with Th1 cells and their effects on bacterial binding and epithelial cell damage. These observations suggest that a "protective" response to vaccines will induce Tr1 cells, or related regulatory Th cells, that are responsible for limiting inflammation. How these cells are derived is unknown and their role in host defense has yet to be defined. This background leads to our general hypothesis that a relative imbalance in helper T cell subsets favors the stimulation of inflammation and epithelial damage in response to persistent infection with H. pylori. More specifically, oxidative stress associated with H. pylori infection selects for "pathogenic" Th1 responses that contribute to epithelial damage while a regulatory "protective" Th1 cell will favor tissue integrity and immunity. The overall objective is to define the T cell response to natural infection with H. pylori or immunization and elucidate the mechanisms governing lymphoepithelial cell interactions in disease versus immunity. This will be achieved in the following Specific Aims: 1). Define the factors selecting for "pathogenic" Th cells associated with H. pylori infection. 2). Identify T cell markers that are correlates of immunity. 3). Define mechanisms of host defense attributed to "protective" Th cells.

Grant: 2R01DK052413-06A1
Program Director: HAMILTON, FRANK A.
Principal Investigator: SCHAUER, DAVID B PHD
Title: H. hepaticus: the pathogenesis of IBD
Institution: MASSACHUSETTS INSTITUTE OF CAMBRIDGE, MA
TECHNOLOGY
Project Period: 1998/01/01-2007/06/30

DESCRIPTION (provided by applicant): Idiopathic inflammatory bowel disease (IBD) is a debilitating condition with no known etiology. The pathogenesis of IBD has been studied using targeted gene mutant (knockout) mice that develop chronic intestinal inflammation, which resembles human IBD. It has been shown in several of these knockout mouse models, including T cell receptor alpha beta (TCR alpha beta), interleukin-2 (IL-2), and IL-10 knockout mice, that germ-free conditions protect against the development of IBD. It does not appear that resident intestinal microbiota are sufficient to trigger disease in these animals, but experimental infection with an emerging group of murine bacterial pathogens called enterohepatic *Helicobacter* species is sufficient to cause IBD. To better understand the pathogenesis of disease in these models, we propose to elucidate the mechanisms by which enterohepatic *Helicobacter* species cause IBD in knockout mice. Although the adaptive immune system is important in the pathogenesis of IBD, knockout mice lacking adaptive immunity are also susceptible to *Helicobacter*-associated IBD. For this reason, our proposed studies focus on interactions between enterohepatic *Helicobacter* species and the innate immune system. Studies will be carried out with *Helicobacter hepaticus*, the most well characterized enterohepatic *Helicobacter* species. We and our collaborators have recently determined the complete genome sequence of *H. hepaticus* ATCC 51449. Taking advantage of the genome sequence, we will identify and characterize candidate bacterial virulence determinants, and generate isogenic mutant strains to test in culture with murine intestinal epithelial cell monolayers and murine macrophages, and in vivo with selected knockout mouse models. These studies will test the hypothesis that discrete bacterial virulence determinants in enterohepatic *Helicobacter* species elicit proinflammatory responses from the innate immune system, which in the absence of a properly regulated adaptive immune system, lead to IBD. It is hoped that these studies will lead to the development of new strategies for the treatment and the prevention of IBD.

Grant: 2R01DK053347-05

Program Director: HAMILTON, FRANK A.

Principal Investigator: SARTOR, RYAN B MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC

Title: Colitis induced by immune responses to luminal bacteria

Institution: UNIVERSITY OF NORTH CAROLINA CHAPEL HILL, NC
HILL

Project Period: 1998/08/01-2007/12/31

DESCRIPTION (provided by applicant): This proposal is to renew a collaborative grant application which includes Dr. Sartor at the University of North Carolina at Chapel Hill and Dr. Sue Tonkonogy at the College of Veterinary Medicine, North Carolina State University. The etiology of the inflammatory bowel diseases (IBD) is unknown, but both genetic and environmental factors are involved. In the current funding period of this grant we have used genetically engineered murine models to provide convincing evidence that the normal endogenous enteric bacterial flora are essential to the development of chronic colitis, in genetically susceptible rodents. Very importantly, we have demonstrated that all resident enteric bacteria are not equal in their capacities to induce inflammation: some are aggressive (*Enterococcus faecalis* in IL-10 KO mice), some are neutral (*H. hepaticus* in IL-10 $-/-$ mice) and some are protective (*Lactobacillus plantarum* in IL-10 $-/-$ mice). Chronic intestinal inflammation in these models is mediated by activated TH1 lymphocytes which are induced by normal cecal bacteria. We have demonstrated an important role for endogenous IL-10 in downregulating pathogenic responses of antigen presenting cells (APC) and T lymphocyte to normal bacterial components. Our data support the hypothesis that chronic intestinal inflammation in genetically susceptible hosts is the result of an overly aggressive cellular immune response to a subset of ubiquitous luminal bacterial constituents. Genetic susceptibility is determined by defective downregulation of inflammatory responses or defective mucosal barrier function. This clinically relevant hypothesis will be addressed by the following Specific Aims: 1. Determine the mechanisms by which endogenous IL-10 regulates responses of APC to environmentally relevant bacterial constituents and inflammatory mediators; 2) Determine the role of endogenous IL-10 produced by APC in regulating responses of naive and activated T cells to environmentally relevant bacterial antigens; 3) Identify the bacterial species preferentially inducing colitis and pathogenic immune responses in IL-10 deficient mice and protective responses in normal mice. The NIH-funded Core Center for Gastrointestinal Biology and Disease at UNC supports a barrier-intact gnotobiotic rodent facility, allowing the investigators a unique environment to selectively colonize germ-free mice with defined luminal bacterial species. These studies will provide novel insights into the pathogenesis of IBD and open new opportunities for novel therapeutic interventions designed to block induction of the antigen-specific immune response to luminal bacteria.

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Grant: 2R01DK053623-06A1
Program Director: HAMILTON, FRANK A.
Principal Investigator: COVER, TIMOTHY L MD
Title: H. Pylori factors associated with peptic ulcer disease
Institution: VANDERBILT UNIVERSITY NASHVILLE, TN
Project Period: 1997/09/30-2007/04/30

DESCRIPTION (provided by applicant): Although most H. pylori-infected persons never develop upper gastrointestinal symptoms, some develop peptic ulcer disease or gastric malignancies. The factors that determine whether or not peptic ulceration occurs in H. pylori-infected persons are not yet well understood. The hypothesis of the current proposal is that H. pylori strains associated with peptic ulcer disease exhibit genetic characteristics that are different from those of non-ulcerogenic strains. Our previous investigations of this topic have focused on vaeA allelic variation and presence/absence of the eag pathogenicity island as bacterial factors that account for differences in disease outcome among H. pylori-infected persons. However, it seems likely that additional strain-specific H. pylori factors might help to determine whether or not peptic ulceration occurs. In particular, diversity among strains in outer membrane protein expression would be expected to have important consequences for bacteria-host interactions. Several studies have suggested that H. pylori strains expressing BabA (an outer membrane protein that binds Lewis b) are associated with an increased risk for peptic ulcer disease. In addition, we present preliminary data indicating that H. pylori strains containing type I hopQ alleles are associated with an increased risk for peptic ulcer disease compared to strains containing type II hopQ alleles. We describe plans to investigate the expression, structure, and functions of these two outer membrane proteins. The specific aims of this proposal are (i) to investigate the expression and structure of the H. pylori Lewis b-binding adhesin BabA (a prototype for the Hop family of H. pylori outer membrane proteins); (ii) to investigate allelic diversity and expression of H. pylori hopQ. These studies should result in a better understanding of the H. pylori factors that help to determine clinical outcome, and ultimately, may lead to advances in the treatment or prevention of H. pylori-associated human diseases.

Grant: 2R01DK053642-06A1
Program Director: HAMILTON, FRANK A.
Principal Investigator: SACHS, GEORGE MB
Title: Gastric Biology of Helicobacter Pylori
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 1997/09/30-2008/07/31

DESCRIPTION (provided by applicant): *H. pylori* is the only bacterium to inhabit the normal human stomach. As a neutrophile only able to grow between pH 6.0 and 8.0, it has evolved several specialized acid adaptive mechanisms to allow it to thrive in the variable acidic milieu of the mammalian stomach. A vital means of elevation of periplasmic and cytoplasmic pH in acidic external pH is achieved via a pH-gated urea channel, UreI, in the inner membrane that allows urea access to intra-bacterial urease at acidic pH. NH₃ generated internally increases both cytoplasmic and periplasmic pH, but not to levels found at neutral external pH when external pH is below 6.0. Other acid adaptive processes are also activated by the reduction in cytoplasmic pH and/or periplasmic pH. The specific aims are (a) fluorimetric cDNA microarray analysis of the complete genome of *H. pylori* to identify genes whose expression is up regulated in response to several different external pH values between pH 2.5 and 6.2 in the absence and presence of physiological concentrations of urea. Among these genes, those that are also able to regulate pH_{in} will be investigated further. Their increase found on the micro-arrays will be confirmed by use of real time PCR. (b) the relative increase in their gene expression will be correlated with changes of the internal pH and inner membrane potential difference to determine the site of pH regulation of expression (c) to show that increased gene expression is associated with increased protein abundance and to also detect proteins whose levels may rise without an increase in gene transcription, changes in protein levels in response to acid will be assessed using pulse labeling, 2D gel electrophoresis and mass spectrometry. Western analysis and enzyme assay will also confirm increased levels of some of the pH regulatory genes and (d) to show that these pH regulatory processes have a role in acid adaptation, the effect of deletion of each of these genes on internal pH and on growth and survival of *H. pylori* in acid will be determined. These studies should provide further insight as to how *H. pylori* has evolved to colonize the human stomach. These studies may provide targets to allow eradication of this class 1 carcinogen without the use of general antibiotics.

Grant: 2R01DK053664-06A1
Program Director: HAMILTON, FRANK A.
Principal Investigator: GOODMAN, KAREN J PHD
Title: H.pylori Infection in Children on the US-Mexico Border
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX
HOUSTON
Project Period: 2003/05/01-2006/04/30

DESCRIPTION (provided by applicant): The aims of the proposed study are to continue observing the natural history of Helicobacter pylori infection in children of low-income families in El Paso, Texas and Juarez, Mexico who have been followed previously from birth for 12-36 months as part of the Pasitos Cohort Study, which aims to identify factors that influence acquisition and persistence of infection in early childhood. Although it is known that H.pylori infection is associated with factors linked to low socioeconomic status, little evidence to date addresses the influence of environmental factors on the risk of acquiring the infection as opposed to the influence of these factors on the probability of having a persistent infection. The aim of our initial study was to observe the natural history of H.pylori infection during the first three years of life. Evidence suggests that ages 3-6 may be critical in determining whether infection persists throughout childhood into adulthood. Therefore, critical information will be gained from continuing to follow this cohort through six years of age. Specifically, we propose to: extend the original aims of the Pasitos Cohort Study to follow children through six years of age; estimate incidence rates of H.pylori infection in children living on both sides of the Rio Grande in El Paso, Texas and Juarez, Mexico from 3- 6 years of age; estimate rates of elimination of H.pylori infection in children from 3-6 years of age; and estimate the effect of socioeconomic indicators, hygiene, and diet on H.pylori incidence, recurrence, and persistence from 3-6 years of age.

Grant: 2R01DK053713-06A1
Program Director: HAMILTON, FRANK A.
Principal Investigator: SMOOT, DUANE T MD
Title: Differential Transcription Factor Activation by H. pylori
Institution: HOWARD UNIVERSITY WASHINGTON, DC
Project Period: 1997/09/30-2007/03/31

DESCRIPTION (provided by applicant): The discovery of *Helicobacter pylori* (H. pylori) infection has greatly changed our understanding of upper G.I. tract diseases, including peptic ulcer disease and stomach cancer. The world health organization has classified H. pylori as a group one carcinogen. Reactive oxygen species (ROS) are known carcinogens and have been shown to play a role in gastric cancer. Our studies have shown that H. pylori stimulates the generation of ROS within gastric cells. By placing gastric cells into a pro-oxidant state, H. pylori increases the risk of DNA damage from ROS and the development of cancer. Epithelial cells protect themselves from DNA damage by undergoing apoptosis. H. pylori induce apoptosis is associated with activation of both NF-kappaB and p53. Also H. pylori induced apoptosis is associated with stimulation of the CD95/Fas pathway. Our preliminary studies show that the increase in p53 protein after exposure of gastric cells to H. pylori is associated with increased expression of p14 ARF and down regulation of mdm2. The studies planned in this proposal will identify any interactions between p53 mediated apoptosis and CD95/Fas-mediated apoptosis in response to H. pylori. We hypothesize that stimulation of intracellular reactive oxygen species within eukaryotic cells is a major mediator of H. pylori induced cellular injury resulting in oxidant associated DNA damage and apoptosis. We further hypothesize that transcription factors p53 and NF-KappaB are important downstream mediators of ROS induced cellular injury from exposure to H. pylori. Our more recent studies show that the rise in p53 after exposure to H. pylori is secondary to stimulation of ARF which stabilizes p53 by preventing Mdm2 inhibition of p53 activity and Mdm2 mediated degradation of p53. Therefore, ROS may be responsible for stimulation of ARF resulting in increased p53. The specific aims of this proposal are: (1) to determine whether or not ROS leads to activation of NF-KappaB which may sensitize gastric cells to apoptosis, dependent or independent of p53; (2) To elucidate mediators of ARF activation by H. pylori leading to increased p53 protein and apoptosis; (3) To determine the involvement of the CD95 (Fas/Apo-1) receptor/ligand system in p53 sensitization of gastric cells to apoptosis induced by H. pylori. Identification of transcription factors activated by this bacterium and elucidation of apoptotic pathways involved will assist us to develop better treatment strategies to prevent serious disease from this infection. By protecting gastric cells from ROS, one may be able to negate the carcinogenic properties of this bacterium.

Grant: 2R01DK054411-06
Program Director: DOO, EDWARD
Principal Investigator: GANDHI, CHANDRASHEKHAR R PHD
Title: ROLE OF STELLATE CELLS IN LIVER FAILURE
Institution: UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PA
PITTSBURGH
Project Period: 1998/09/25-2007/08/31

DESCRIPTION (provided by applicant): Acute liver failure is a devastating clinical condition with a high mortality rate. This affects patients with and without prior liver disease. Inability of hepatocytes to regenerate and their progressive death leads to acute liver failure. The mechanisms of hepatocyte depletion in acute liver failure are inadequately understood, precluding effective pharmacological therapy. However, endotoxemia is always associated with acute liver failure and is a major contributor of catastrophic failure. In the United States alone more than 20,000 people die of septic shock and evidence indicates that hepatic stellate cells inevitably play a major role in response to endotoxemia. The normally quiescent stellate cells regulate liver blood flow by contractility and maintain its architecture by producing components of extracellular matrix. During chronic liver injury, stellate cells transform into proliferating, highly contractile and fibrogenic myofibroblast cells. Endotoxin causes upregulation of the endothelin system in stellate cells and the consequent profound hepatic vasoconstriction contributes to endotoxin-induced liver injury. Moreover, endotoxin-conditioned media of quiescent and activated stellate cells strongly inhibit DNA synthesis in hepatocytes. Endotoxin increases the expression of nitric oxide, IL-1-beta, IL-6, but not of TGF-beta or TNF-alpha in both phenotypes. However, none of these potent mediators is responsible for the inhibition of hepatocyte DNA synthesis. Also, stellate cells in the presence of endotoxin and reactive oxygen species release factors which kill hepatocytes. Reactive oxygen species are generated by the resident macrophages (Kupffer cells) in response to endotoxin. These observations strongly suggest the synthesis of one or more as yet unidentified mediators which promote liver failure. The specific aims of this proposal are: 1) to determine the cellular mechanisms by which stellate cells respond to endotoxin, 2) to determine the mechanisms by which stellate cells plus endotoxin- or stellate cells plus reactive oxygen species-conditioned media cause injury to hepatocytes, 3) to determine the effects and mechanisms of endotoxin treatment of normal and cirrhotic rats on stellate cell-mediated hepatocyte injury, and 4) to identify and characterize the mediators of hepatocyte injury produced by stellate cells. The outcome of this investigation will provide profound insights into the central regulatory role of stellate cells in liver pathophysiology. Importantly, identification of the stellate cell-derived mediators will provide a rational basis for the therapy of life-threatening liver failure and other liver disorders.

Grant: 2R01DK055828-06A1
Program Director: MULLINS, CHRISTOPHER V.
Principal Investigator: SABAN, RICARDO DVM
Title: LPS-Peptide Interaction in Bladder Inflammation
Institution: UNIVERSITY OF OKLAHOMA HLTH SCIENCES CTR OKLAHOMA CITY, OK
Project Period: 1998/09/29-2008/01/31

DESCRIPTION (provided by applicant): This proposal is a competing continuation of a four year grant which seeks to expand upon the role of mast cells and mast cell products, substance P (SP), and LPS in bladder inflammation and further elucidate the molecular pathways resulting in up-regulation of inflammatory genes. The original specific aims were to determine the role of neutral metalloendopeptidase (NEP), SP receptors, and mast cells in the development of cystitis, utilizing NEP $-/-$ mice, NK-1R $-/-$ mice, and mast cell deficient mice (kit[w]/kit[w-v]), and those which have been reconstituted with mast cells by adoptive transfer from normal mice and NK-1R $-/-$ mice. In the present proposal, we seek to expand upon the hypothesis that regardless of the stimuli there is a common intracellular activation pathway of bladder inflammation. We now include the possible role of Protease Activated-Receptors (PAR) as a signaling mechanism owing to tryptase release following mast cell degranulation. There are 6 specific aims in this proposal seeking to determine the role of PARs in the development of acute and chronic bladder inflammation. We will employ methodology and animal models published by this laboratory, including determination of cellular pattern of the 4 different PAR proteins and their gene expression by immunohistochemistry and in situ hybridization. Results will be confirmed by western blotting and quantifiable RNase protection assay developed by this laboratory. The role of PAR in inflammation will be confirmed in PAR $-/-$ mice. In addition, the role of PARs 1 and 2 on mast cells will be determined by examining kit[w]/kit[w-v] mice, which are deficient in mast cells, after they receive bone-marrow mast cells from PAR1 $-/-$ or PAR2 $-/-$ mice. This study seeks to determine the molecular pathway of acute and chronic bladder inflammation. Information generated by this study should elucidate whether bladder inflammatory responses, regardless of etiology, occur via a common mechanism or whether the endpoints are specific for each stimulus.

Grant: 1R01DK058928-01A2
Program Director: HAMILTON, FRANK A.
Principal Investigator: KOVBASNJUK, OLGA N PHD
Title: Shiga toxin 1:uptake mechanisms and intracellular action
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 2003/09/15-2006/08/31

DESCRIPTION (provided by applicant): Foodborne diarrheal disease cause 76,000,000 cases each year in the United States. Intestinal pathology caused by Shiga toxin-producing Escherichia coli (EHEC), important food-borne pathogens, includes approximately 35% of all bloody diarrhea in the United States, an unknown % of watery diarrhea, and the life-threatening systemic manifestations (observed in up to 10% of cases) of infection, the hemolytic uremic syndrome (HUS) and encephalopathy. EHEC are the leading cause currently of acute and chronic renal failure in children in the USA. EHEC is a particularly worrisome foodborne pathogen, because the number of outbreaks caused by EHEC continue to significantly increase and there is no effective specific therapy for this illness which is lethal in up to 10% of children who develop HUS. Thus, there is a great need to better understand the pathogenesis of this infection to promote development of new therapeutic approaches to treat EHEC infection and its complications. Understanding the complex mechanism of Stx uptake and trafficking pathways into colonic epithelium may help to identify new drug targets and develop new strategies directed at preventing toxin action on human intestine. In this application we will study the mechanism of Stx1 and its B-subunit (Stx1B) uptake, trafficking and intracellular action using animal and human intestinal epithelial cell models. In Aim 1 we will study the role of lipid rafts (LR) in Stx1/Stx1B translocation across the apical cell surface. We propose to test the role of LR proteins, which are associated with toxin receptor in toxin translocation machinery. In Aim 2 we will study a newly recognized Stx1/Stx1B internalization pathway from plasma membrane into the nucleoli. In Aim 3 we will study the mechanisms of Stx1/Stx1B-induced apoptosis and its role in toxin-related intestinal inflammation. These proposed studies should provide new insight into the understanding of the molecular basis of Stx-mediated intestinal epithelial cell injury and facilitate the design of strategies to prevent Stx1 uptake and intracellular action, and thus serve to find new targets to interfere with EHEC-related gastrointestinal pathophysiology.

Grant: 1R01DK059911-01A2
Program Director: HAMILTON, FRANK A.
Principal Investigator: LORENZ, ROBINNA G
Title: T Cell Initiated Gastric Pathology
Institution: UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL
BIRMINGHAM
Project Period: 2003/01/10-2007/12/31

DESCRIPTION (provided by applicant): The bacteria *Helicobacter pylori* is a major pathogen which, in addition to infecting over half of the world's population, is linked to gastric and duodenal ulcer disease, mucosal-associated lymphomas, and adenocarcinoma. Infection with *H. pylori* results in an early gastric infiltration of neutrophils, macrophages, CD4+ T cells, and B cells. These gastric infiltrates are accompanied by increased levels of gastric IFN-gamma, TNF-alpha, and IL-1 and by loss of gastric parietal cells, zymogenic cells, and dysplasia of gastric mucosal cells. Very little is known about the mechanisms by which this gastric infiltrate induces subsequent gastric epithelial pathology. A small animal model of *Helicobacter* infection, the *H. felis* mouse model, closely mimics the human disease in that severe gastric atrophy and gastric adenocarcinoma develops after infection. This model has allowed a careful analysis of the adaptive immune response to *Helicobacter* infection. Using a novel adoptive transfer model of disease, we have shown that it is the host CD4+ T cell response which is crucial for the development of *H. felis*-associated gastric pathology. This has directed our attention to the role of the CD4+ T cell, and its potential effector mechanisms, in the development of *Helicobacter*-associated gastric epithelial cell destruction and pathology. This grant application focuses on the hypothesis that recruitment and activation of CD4+ T cells in the stomach results in secondary non-antigen specific gastric epithelial cell alterations. These changes in epithelial cell proliferation and differentiation lead to gastric dysplasia and cancer formation. In order to elucidate this immune/ epithelial cell relationship and its sequelae, we propose to: 1) characterize the antigen recognition requirements of the CD4+ T cell critical for the development of *Helicobacter*-associated gastric pathology; and 2) determine the role of secreted or cell-surface products in the generation of *Helicobacter*-associated gastric epithelial pathology. These studies will utilize both in vivo models of disease, as well as a novel primary gastric epithelial cell culture system. The understanding of the basic mechanisms by which the host immune response to *Helicobacter* induces gastric epithelial pathology will lay the foundation for further studies on the regulation of the inflammatory response and the design of immunotherapies for *Helicobacter* infection and associated digestive diseases.

Grant: 1R01DK060606-01A2
Program Director: SERRANO, JOSE
Principal Investigator: ZHANG, JIAN X PHD
Title: Prostanoids and Liver Microcirculation in Stresses
Institution: UNIVERSITY OF NORTH CAROLINA CHARLOTTE, NC
CHARLOTTE
Project Period: 2003/06/01-2006/03/31

DESCRIPTION (provided by applicant): Endotoxemia is a common but severe complication of cirrhosis frequently causing liver injury and even organ failure. The mechanism underlying the increased susceptibility of the cirrhotic liver to endotoxemia in the sequential stresses, however, is not completely understood. Studies have shown a hepatic upregulation of constrictor endothelin (ET) and a decreased release of vasodilator nitric oxide (NO) in the cirrhotic liver. Our preliminary studies have shown that in cirrhosis, constrictor prostanoids are released in response to ET, and the action of the prostanoids is modulated by NO. We also showed that endotoxemia as a secondary stress caused an additional upregulation in already increased ET gene expression in the cirrhotic rat liver, but endotoxin-induced expression of inducible nitric oxide synthase was blunted by the preexisting cirrhosis. We therefore hypothesize that liver cirrhosis as a pre-existing condition primes the hepatic microcirculation for predisposition to an imbalance between constrictor and dilator influences by sensitizing the ET/constrictor prostanoids pathway and decreasing production of NO. We further hypothesize that endotoxemia as a secondary stress further activates the pathway leading to dysregulation of the hepatic microcirculation and ultimately hepatocellular injury. To test these hypotheses, three specific aims are proposed: 1) determine whether cirrhosis as a pre-existing condition primes the hepatic microcirculation for predisposition of an imbalance between constrictor and dilator influences by sensitizing the ET-mediated release of vasoconstrictor prostanoids; 2) determine whether endotoxemia as a secondary stress enhances the pressor response mediated by the release of vasoconstrictor prostanoids in response to ET; 3) determine whether an overwhelming increase in ET accompanied by the sensitization of ET-induced release of prostanoids and an attenuated expression in iNOS following the sequential stresses result in an imbalanced regulation of vasoconstriction and vasodilation and dysregulation of the hepatic microcirculation. This proposal not only will allow us to evaluate the role of constrictor prostanoids in regulation of hepatic microcirculation in cirrhosis and endotoxin-induced sequential stresses, but also will provide invaluable information on therapeutic strategies to prevent hepatic microcirculatory failure under the double stressed conditions.

Grant: 1R01DK061315-01A1
Program Director: SERRANO, JOSE
Principal Investigator: ROTH, ROBERT A PHD OCCUP
HEALTH:TOXICOLOGY
Title: INFLAMMATION AND DRUG IDIOSYNCRASY
Institution: MICHIGAN STATE UNIVERSITY EAST LANSING, MI
Project Period: 2003/09/01-2007/07/31

DESCRIPTION (provided by applicant): "Drug idiosyncrasy" refers to a toxic response to a drug that occurs in a small fraction of people and bears no obvious relationship to dosing regimen. Numerous drugs developed for various therapeutic purposes have produced in people idiosyncratic responses that have resulted in serious injury to liver and other organs. These reactions typically do not become apparent in preclinical animal studies, and little is understood about underlying mechanisms. Animal models with the potential to enable prediction/early identification of idiosyncratic responses could prevent human suffering and lead to understanding of mechanisms. In preliminary studies, we have found in rats that modest inflammation produced by a small, nontoxic dose of endotoxin (LPS) can render an otherwise nonhepatotoxic drug hepatotoxic. For example, in rats given a nontoxic dose of chlorpromazine, co treatment with a small dose of LPS resulted in liver injury and elevated plasma creatine kinase activity, two responses that occur idiosyncratically in people during therapy with this and related drugs. Similarly, a nontoxic dose of LPS; can render ranitidine hepatotoxic in rats and mice. These preliminary results suggest a novel mechanism for drug idiosyncrasy and raise the possibility of creating useful animal models for such responses in humans. The hypothesis to be tested is that idiosyncratic drug reactions that occur in humans can be reproduced in animals by drug administration during a concurrent episode of mild inflammation. Several drugs that have caused idiosyncratic liver injury in humans (chlorpromazine, ranitidine, flutamide) and two that have not (promethazine, famotidine) will be used. Rats will be co exposed to a drug and to a dose of LPS that causes "modest inflammatory response" to determine if the co treatment reproduces the idiosyncratic drug responses that people experience. Dose-response and temporal relationships will be defined. Inflammatory factors (e.g. neutrophils, tumor necrosis factor-alpha, cyclooxygenase 2) likely to be critical to the toxic response will be evaluated. In addition, a cell-based, in vitro system will be developed and used to explore intracellular signaling mechanisms that enable drugs to interact with inflammatory factors to result in synergistic hepatocyte killing. Results from these studies will be an important step toward creating predictive animal models of human drugs idiosyncrasy and exploring underlying mechanisms.

Grant: 1R01DK061410-01A1
Program Director: MAY, MICHAEL K.
Principal Investigator: MERCHANT, JUANITA L
Title: Altering Gastric Epithelial Cell Differentiation
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2003/02/01-2008/01/31

DESCRIPTION (provided by applicant): Chronic inflammation in the stomach (gastritis) is usually associated with *Helicobacter pylori*, but may occur from bacterial overgrowth because of hypochlorhydria. Chronic gastritis also results in initial increase then loss of parietal cells over time (chronic atrophic gastritis). A recurring theme is that disruption of parietal cell function eventually results in fewer parietal cells followed by an expansion of the mucous and undifferentiated cell types in the stomach. Interestingly, destruction of the parietal cell through ectopic expression of toxins has also been reported to generate the same phenotype. In some instances, these phenotypic alterations progress to the point where mucous cell types emerge, a subset of which express intestine-specific genes (intestinal metaplasia). Intestinal metaplasia is a condition that predisposes the gastric mucosa to cancer. Central to initiating these important alterations are changes in the parietal cell population. In this proposal, we hypothesize that an important trigger altering the normal phenotypic pattern of gastric epithelial cells is inflammation generated from bacterial colonization. The primary goal of this proposal is to understand how components of a bacterial infection trigger parietal cell atrophy and subsequently pre-neoplastic changes. The preliminary results show that both CagA and INF γ alter gastric architecture. First, the experiments proposed use a transgenic mouse model expressing CagA (Aim 1) or treatment of mice with pro-inflammatory cytokines (Aim 2) to alter parietal and mucous cell populations. Second, in vitro studies in primary parietal and mucous cells cultures, will be used to dissect the signaling pathways activated (Aim 3) and will study the target proteins regulated during the transformation of the mucosa from chronic atrophy to dysplasia (Aim 4). We will examine whether Sonic hedgehog expressed primarily in parietal cells may be lost during parietal cell atrophy and contribute to the increase in mucosal proliferation and subsequently transformation. These studies will further our understanding of how corpus atrophy predisposes the gastric mucosa to neoplastic transformation.

Grant: 1R01DK061417-01A1
Program Director: MAY, MICHAEL K.
Principal Investigator: GEWIRTZ, ANDREW T BS
Title: Flagellin-induced gut epithelial chemokine secretion
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 2003/03/15-2008/02/29

DESCRIPTION (provided by applicant): The active flares of colitis (collectively referred to as inflammatory bowel disease [IBD]) are characterized by migration of neutrophils to the intestinal lumen forming an intestinal crypt abscess. Such neutrophil movement is directed via polarized chemokine secretion by the epithelial cells that line the intestine. As I transitioned from trainee to faculty, I elucidated the mechanism by which such epithelial chemokine secretion is regulated. Briefly, I found that epithelial Ca^{++} mobilization is a key signal regulating such chemokine secretion. Further, such chemokine secretion is activated via the protein flagellin that is secreted by bacteria. Flagellin is secreted by commensal and pathogenic bacteria. However, only flagellin that crosses epithelia to the basolateral membrane domain activates epithelial chemokine secretion. Such translocation of flagellin is mediated by pathogens, but not commensal bacteria, explaining why, normally, only pathogenic bacteria induce epithelia to orchestrate inflammation. However, aberrant translocation of, and/or responses to, flagellin may occur in IBD and thus may underlie the inappropriate chemokine secretion that occurs in IBD. For my first independent award application, I propose to expand on the above studies while investigating the hypothesis that bacterial-epithelial interactions regulate the neutrophil infiltration (i.e., active inflammation) associated with both innate immunity and IBD. Consequently, pharmacologic manipulation of these interactions can be therapeutic for IBD. This hypothesis will be studied in vitro and in vivo via three specific aims, all of which are supported by substantial preliminary data. Specifically, I will characterize bacterial translocation of flagellin across intestinal epithelia, investigate how flagellin activates epithelial chemokine secretion, and further define the activation of anti-inflammatory signaling pathways by lipoxins. In vitro and in vivo models will be utilized.

Grant: 1R01DK063008-01
Program Director: HAMILTON, FRANK A.
Principal Investigator: ZHANG, QIJING PHD
Title: Mechanisms of antibiotic efflux in *Campylobacter*
Institution: IOWA STATE UNIVERSITY AMES, IA
Project Period: 2003/04/01-2006/03/31

DESCRIPTION (provided by applicant): *Campylobacter jejuni*, an important foodborne pathogen causing gastroenteritis in humans, has evolved multiple mechanisms to counteract the action of various antibiotics, which has posed a serious threat to public health. Many of these resistance mechanisms, such as *gyrA* mutations and beta-lactamase production, confer *Campylobacter* resistance to specific antibiotics. However, the active efflux systems, which extrude structurally diverse antibiotics out of bacterial cells, contribute to the intrinsic and acquired resistance to multiple drugs. Although previous studies suggested the possible presence of functional efflux systems in *C. jejuni*, the antibiotic efflux machinery in this pathogen has not been defined. Using transposon mutagenesis in conjunction with other approaches, we have recently characterized a three-gene operon (named *cmeABC*) encoding a tripartite antibiotic efflux pump that contributes to *C. jejuni* resistance to structurally unrelated antibiotics, heavy metals, bile salts, and other toxic compounds. Our preliminary data and the genomic sequence of *C. jejuni* NCTC 11168 suggested the presence of an additional antibiotic efflux system (name *cmeDEF*) and the possible regulation of *cmeABC* and *cmeDEF* by transcriptional repressors. Based on these observations and the known features of bacterial antibiotic efflux systems, we hypothesize that *CmeDEF* in conjunction with *CmeABC* plays an important role in extruding various agents, and the modulated expression of the efflux pumps by regulatory proteins contributes significantly to the intrinsic and acquired resistance of *Campylobacter* to multiple antimicrobials. To test our hypothesis, we plan to i) determine the role of *CmeDEF* and its interplay with *CmeABC* in mediating *Campylobacter* resistance to multiple drugs and ii) to identify and characterize the transcriptional repressors that modulate the expression of the antibiotic efflux systems. Various genetic and biochemical approaches, including random and site-specific mutagenesis, recombinant proteins, substrate accumulation assay, and DNA binding assays will be utilized to define the functions of the efflux systems and their interplay with regulatory proteins. It is anticipated that the proposed studies will close a major gap in our understanding of the antibiotic resistance mechanisms in *C. jejuni* and may open new avenues for the design of effective means to prevent and treat antibiotics-resistant *Campylobacter*.

Grant: 1R01DK063041-01
Program Director: KARP, ROBERT W
Principal Investigator: BERG, DOUGLAS E PHD
Title: H.PYLORI POPULATION GENETICS AND GENOME EVOLUTION
Institution: WASHINGTON UNIVERSITY ST LOUIS, MO
Project Period: 2003/06/01-2008/05/31

DESCRIPTION (provided by applicant): *Helicobacter pylori* (Hp) are one of the most genetically diverse of bacterial species. Our preliminary DNA-based phylogenetic analyses showed that genotypes of Hp in Spain differ from those in East Asia, and that genotypes of Hp from Peruvians in Lima are mostly Spanish-like. The studies proposed for this grant period have four main specific aims. AIM 1. To better understand Hp genetic diversity and genome evolution. We will extend current phylogenetic studies to Hp strains from other Amerind people in Latin America, to test predictions that most such Hp strains will also be Spanish-like at most loci. Any Hp population found to be rich in alleles unlike those of Spain will be examined further. Hp from elsewhere in Europe, Asia and Africa will also be studied (i) to better understand Hp population genetic structure worldwide, (ii) test predictions that Hp gene pools in Japan and Spain represent subsets of more genetically diverse East Asian and European gene pools, respectively. AIM 2. To identify naturally occurring allele differences that affect fitness and understand their basis. We will test the generality of our finding that East Asian-type alleles of the *comB9* gene are abundant in Hp from Amerinds in Lima, and test if this reflects selection. More generally, additional cases of unusual abundance of particular alleles or genes will be investigated to identify bacterial traits important in specificity of host interaction. Effects on fitness of candidate genes or alleles will be examined using genetically manipulated rodent-adapted Hp strains and mouse and gerbil infection models. AIM 3. To examine Hp evolutionary dynamics within family units, Hp strains from parents and children in individual Peruvian families will be genotyped to gain insights into relatedness, intrafamilial vs. community transmission, and mutation, recombination, and selection on Hp in societies where overall risks of infection are high. AIM 4. To detect transmission between communities, mixed infection, and potential for recombination. Hp from Japanese Peruvians will be genotyped to detect (i) retention of ancestral Japanese-type genes, vs. community acquisition of putative Native Peruvian (Spanish-type) strains; (ii) mixed infections, especially involving strains of different ethnic origins, (iii) extent of recombination in a brief time frame, and (iv) selection for particular genes or alleles.

Grant: 1R01DK063250-01
Program Director: MULLINS, CHRISTOPHER V.
Principal Investigator: WELCH, RODNEY A
Title: D-serine/DsdCXA Control of E. coli Uropathogenesis
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 2002/12/13-2007/11/30

DESCRIPTION (provided by applicant): Escherichia coli is the most common cause of community acquired urinary tract infection (UTI) and a leading cause of nosocomial UTIs and sepsis. There are an estimated 8 million physician visits per year in the U.S for UTIs with significant associated morbidity and expense (> \$1,000,000,000). We tested the hypothesis that virulence genes responsible for the pathogenesis of life-threatening E. coli extraintestinal infections, such as pyelonephritis and sepsis can be identified by comparison of the genome sequence of urosepsis E. coli strain CFT073 to either E. coli laboratory strain MG1655 or O157:H7 strain EDL933. We identified >300 CFT073-specific loci. The continued study of the region surrounding the D-serine deaminase genes (dsdCXA) is especially compelling. An allelic knockout mutant of dsdA, that encodes D-serine deaminase, is unaltered in expression of type 1 pili-mediated adherence, but 300-fold more competitive than the wild type strain in colonizing the bladder or kidney of mice infected in an ascending model of UTI. DsdC is a positive effector of dsdXA transcription and a member of the lysR-family of regulators. By in vivo and in vitro gene expression techniques we will test the hypotheses that D-serine through interaction with either dsdC or other co-effectors affects expression of multiple genes that directly influence CFT073 pathogenesis in murine models of disease. We will also identify environmental conditions and additional genes that affect the expression of the dsdCXA genes. Two such gene candidates are ipuAB (integrase-like proteins of uropathogens) that are immediately adjacent to the dsdCXA genes in the chromosome of CFT073 as well as other uropathogenic E. coli. ipuAB are homologs of the type 1 pili phase-switch recombinases, fimB and fimE that are linked to and control expression of the E. coli type 1 pilus fim operon. We will test the hypothesis that these genes provide an additional phase-switch system that controls expression of dsdCXA or other unknown genes. The objective of the proposed project is to identify and characterize critical virulence genes for E. coli involved in serious human diseases. This information will be of use for the development of new chemotherapeutic and vaccine strategies.

Grant: 1R01DK066079-01
Program Director: MULLINS, CHRISTOPHER V.
Principal Investigator: LUO, YI MS
Title: BCG in Endogeneous and Exogenous Antigen-Induced T Cell
Institution: UNIVERSITY OF IOWA IOWA CITY, IA
Project Period: 2003/09/30-2006/07/31

DESCRIPTION (provided by applicant): The purpose of this project is to create antigen-induced T helper (Th) polarized bladder inflammation models to mimic the postulated pathogenesis of interstitial cystitis (IC) and investigate the potential effect of the Bacillus Calmette-Guerin (BCG) vaccine on treating the disease in these models. The long-term goal is to develop effective treatment strategies to combat IC. Towards achieving these goals, three specific aims will be undertaken: Specific Aim 1: To create an exogenous antigen-induced bladder inflammation model to determine the role of systemic Th1 and Th2 immune polarization in the development of bladder inflammation. To create this model, T helper cells will be isolated from transgenic mice (termed OT-II) that express Th cells specific for the ovalbumin (OVA) antigen. These Th cells will be differentiated in the laboratory into Th1 or Th2 subsets, and adoptively transferred into genetically compatible mice. Cystitis will be induced in the recipient mice by instilling OVA directly into the bladder. This model will particularly lend itself to studies on acute inflammation. Specific Aim 2. To establish a bladder autoimmune disease model by creating a novel transgenic mouse strain that expresses OVA as a "self" antigen via promoter-specific production by the urothelium and to determine the role of the Th immune polarization in the development of this disease. This transgenic mouse strain (termed URO-OVA) will be genetically engineered with DNA containing a bladder-specific promoter and the gene for OVA. An autoimmune form of cystitis will be developed in these URO-OVA mice by adoptive transfer of polarized OT-II Th cells. This model particularly lends itself to studies on chronic inflammation. Specific Aim 3. To assess the therapeutic effect of BCG in antigen-induced bladder inflammation. BCG will be evaluated for its effect on treating Th2 polarized cystitis (the hypothesized type responsible for IC) in both exogenous and endogenous antigen-induced cystitis models. BCG is predicted to shift the diseased Th2 immune state back to a favorable Th1 state. Successful completion of this study will establish the pathological and immunological characteristics for both Th1 and Th2 type cystitis, shed light on BCG's effect on treating this disease, and aid in the future development of prevention and treatment strategies for IC and other painful bladder conditions.

Grant: 1R01DK066101-01
Program Director: MULLINS, CHRISTOPHER V.
Principal Investigator: SABAN, RICARDO DVM
Title: Bladder transcriptome in experimental inflammation
Institution: UNIVERSITY OF OKLAHOMA HLTH SCIENCES CTR OKLAHOMA CITY, OK
Project Period: 2003/09/30-2008/07/31

DESCRIPTION (provided by applicant): Interstitial Cystitis (IC) is a chronic bladder syndrome characterized by urinary urgency, frequency, nocturia pain and sterile urine. Although inflammation is not a universal characteristic of biopsies from IC patients, it seems that inflammation underline all major bladder pathologies including malignancy and represents a defense reaction to injury caused by physical damage, chemical substances, microorganisms or other agents. Indeed, areas of bladder inflammation are found in bladder disease including carcinoma, during chronic implantation of catheters, and an integrative part of bladder responses to intravesical Bacillus Calmette-Guerin (BCG). Although BCG has been proposed as a promising option for treatment of IC its mechanisms of action is not completely known. One of the theories is that intravesical BCG may be effective in treating by correcting an aberrant immune imbalance in the bladder, leading to long-term symptomatic improvement. It remains to be determined the nature of this immune imbalance. As nuclear factor kappaB (NFkappaB) has been described to modulate both bladder inflammation and the effects of BCG therapy, it is fair to propose, as a central hypothesis, that intravesical BCG leads to activation of NFkappaB and translation of pro- and anti-inflammatory genes that control both the immune and inflammatory system. The long-range goals of this application are to determine the gene networks involved in bladder responses to BCG therapy. To meet these goals, we will use the mouse as a vehicle for understanding basic biological questions regarding BCG and to permit rigorous control of experimental design. Transgenic mice with reporter genes expressed specifically on endothelial cells (Tie2-LacZ) and on promoter elements responsive to NFkappaB (p105-LacZ and p65-LacZ) will be used to address our hypothesis. In addition, the appropriate use of micro array technology combined with subtractive hybridization (SSH) will identify key molecules and mechanisms involved in the transition between acute and chronic inflammation in individual bladder layers. To address our central hypothesis, Aim 1 will quantify the time course alterations in bladder morphology as a consequence of acute and chronic intravesical BCG therapy. Aim 2 will test the hypothesis that NFkB plays a central role on BCG-induced bladder inflammation and Aim 3 will test the hypothesis that acute and chronic instillation of BCG induce a differential gene regulation in bladder mucosa and detrusor muscle. For this purpose, we will use micro array technology combined with suppression subtractive hybridization (SSH) to select bladder mucosa- and detrusor specific genes. Following target validation of SSH-selected genes, a custom array will be developed. This micro array will be used to determine how the bladder transcriptome is altered by BCG therapy.

Grant: 1R01DK066349-01
Program Director: MULLINS, CHRISTOPHER V.
Principal Investigator: BJORLING, DALE E DVM
Title: Neurovascular and Behavioral Response to Cystitis
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 2003/09/30-2008/07/31

DESCRIPTION (provided by applicant): Interstitial Cystitis (IC) is a disorder of uncertain etiology that primarily results in increased supra-pubic pain and increased voiding frequency. Although IC often appears to develop in the wake of urinary tract infection (acute or repeated), the acute and chronic effects of urinary tract infection on bladder innervation, vasculature, and function have not been well characterized. Most studies of inflammatory cystitis in animal models have utilized chemically induced models of cystitis and focused on acute effects. There is a critical need to extend observations made on chemically induced cystitis to the effects of bacterial cystitis and to characterize the long-term effects of acute and chronic infectious cystitis. The proposed research will address the hypothesis that repeated urinary tract infection results in persistent changes in bladder innervation, vasculature, and function that contribute to symptoms of IC after the infection is apparently resolved. We will also address the corollary hypotheses that these changes may be mimicked by prolonged chemical cystitis induced by systemic administration of CYP and that susceptibility of mice to the durable effects of inflammatory cystitis on bladder innervation, vasculature, and function vary among different strains of mice. The proposed research will use an integrated experimental approach and novel imaging techniques to characterize the cellular, histological, neurological, and behavioral response to bladder inflammation (acute and chronic) and determine whether changes persist after removal of the initiating stimulus. This research will investigate the durability of changes that occur in response to a common clinical problem and elucidate the ways in which a "simple UTI" may create a cascade of changes in the bladder physiology and function, neuroregulation, pain perception and behavior that underlie the complex clinical face of IC. The long range plan for this line of research is to link the information generated in the proposed study to future investigations into the genetic basis for variations in susceptibility to cystitis among strains of mice and how this relates to variability among humans regarding sensitivity to the effects of UTI and the occurrence of painful bladder disorders of uncertain etiology, such as IC.

Grant: 1R03DK062149-01A1
Program Director: ROBUCK, PATRICIA R.
Principal Investigator: SNYDER, JOHN D MD
Title: Validation of Non-invasive Diagnosis of Hp
Institution: UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA
Project Period: 2003/06/01-2005/05/31

DESCRIPTION (provided by applicant): H. pylori causes gastritis, duodenal ulcers and is strongly linked to gastric cancer, but most infections are asymptomatic. No group of GI signs and symptoms have been shown to predict infection or associated disease. At present, no validated non-invasive tests exist to diagnose active H. pylori infection in children. Despite this, many clinicians test for H. pylori in children with gastrointestinal (GI) symptoms and will treat if the infection is "found". The urgent need for a rational approach to treatment of H. pylori in children is underscored by the fact that more than 11 million U.S. children are infected, using a conservative prevalence estimate of 15%. A randomized controlled trial of the effect of H. pylori eradication on GI symptoms in children is clearly needed. Before conducting such a trial, important preliminary validation studies are required. This study will test the overall hypothesis: that it is feasible to use non-invasive tests and a symptom assessment instrument (SAI) to determine the presence/absence of H. pylori infections in children with GI symptoms and can be used as diagnostic surrogates for the more invasive endoscopy and biopsy. Since commercial serology tests are not accurate in children, aim 1 will be to determine the feasibility of using the non-invasive 13C urea breath test and stool antigen test, performed at the time of previously scheduled endoscopy and biopsy, to accurately detect active H. pylori infection in children and thus be considered as potential effective surrogate tests for diagnosis. Preliminary data on the sensitivity, specificity, positive and negative predictive value of these tests will also be compared using the standards for diagnosis established by the FDA. Aim 2 will be to determine the feasibility of using a pediatric gastrointestinal (GI) symptom assessment instrument (SAI), administered at the time of endoscopy, to evaluate GI symptoms of children with symptomatic H. pylori infection. Preliminary data will be obtained on the ability of the SAI to measure the severity of signs and symptoms based on endoscopic and histologic findings.

Grant: 1R21DK062021-01A1
Program Director: NYBERG, LEROY M.
Principal Investigator: LIESKE, JOHN C MD
Title: Role of Nanobacteria in Human Calcifying Disease
Institution: MAYO CLINIC COLL OF MEDICINE, ROCHESTER, MN
ROCHESTER
Project Period: 2003/08/01-2005/07/31

DESCRIPTION (provided by applicant): Inflammation is characteristic of human calcifying diseases, including nephrolithiasis and atherosclerosis. While evidence has linked infectious agents to the inflammation present in atherosclerosis, factors that contribute to the calcification remain to be identified. Preliminary data are presented demonstrating that nanobacteria, a novel, slow-growing microorganism which forms a mineralized shell, can be isolated from diseased, calcified tissues of humans and experimental animals. Therefore, the central hypothesis of this proposal is that nanobacteria are a previously unexpected pathogenic factor in these human diseases. Nanobacteria remain controversial in the scientific community because genetic material has not yet been identified. Therefore, the major goal of this two-year R-21 proposal is to clone and characterize unique nanobacterial DNA sequences in order to unequivocally identify and classify this organism, and to develop definitive genetic tests for evaluating human and experimental tissues. A multidisciplinary team has been created with the long-term goal to isolate nanobacteria from tissue of humans and experimental animals, grow the isolates in culture, and then inoculate experimental animals and cells with the organism to reproduce a calcific response, as required to fulfill Koch's postulates. Distinct advantages of this grant application include the vast experience in Laboratory Medicine for extracting, concentrating, and purifying nucleic acid and developing rapid molecular diagnostic tests for infectious disease agents, as well as the use of cryogenic microcomputer tomography (CT) which allows 3D imaging, histomorphometric/immunological imaging, and genetic probing of a single sample. This is a HIGH-RISK proposal, but if nanobacteria are routinely identified in diseased and calcified renal and atherosclerotic tissue, our understanding of these common human diseases would be revolutionized and HIGH-IMPACT consequences would likely ensue, including new treatment approaches for these and possibly other calcifying diseases.

Grant: 1R21DK062889-01
Program Director: NYBERG, LEROY M.
Principal Investigator: HURST, ROBERT E
Title: Urothelial cell response to bacterial infection
Institution: UNIVERSITY OF OKLAHOMA HLTH SCIENCES CTR OKLAHOMA CITY, OK
Project Period: 2003/05/01-2005/04/30

DESCRIPTION (provided by applicant): The urothelium maintains an active set of defenses against bacterial infection. These include static defenses in the form of surface molecules that inhibit bacterial attachment and active responses to eliminate an established bacterial infection. The long-term aim of this study is to elucidate the mechanisms by which antibacterial defenses are modulated by infection. Our hypothesis is that in an environment of increasing bacterial resistance to antibiotics, understanding the natural mechanisms of defense should permit their augmentation as a means to prevent or treat infection, particularly in vulnerable populations. Enterococcus spp. rank second among the leading cause of bacterial UTIs. The high antibiotic resistance prevalent among this genus makes treatment of enterococcal infections a therapeutic challenge. Previous research in our laboratory has established models for urothelium grown in 3 dimensions that closely mimics both the morphology and functional genomics of the bladder urothelium. In this model system, the environment of the urothelial cells can be controlled precisely and specific modulatory proteins can be added, or the actions of specific genes can be inhibited or augmented in a realistic cell culture environment. We will ask two broad questions: 1. How does the urothelium respond to bacterial infection? 2. Can altering expression of specific genes in the urothelium modulate the response to bacterial infection? This approach will integrate closely with independent bacterial and animal model studies carried out by other members of this collaborative research group. Our model permits mechanistic investigations in which the role of specific molecules in the response of urothelium to infection can be tested. Our main objective for this period of support is to expand on current cancer-related cDNA array technology in our laboratory to investigate genome-wide changes in the expression of human urothelial cells in 3-dimensional culture as they experience infection with enterococcus. We plan to use the Clontech system on plastic consisting of over 8,000 named human genes. The aims are to cluster genes according to their behavior over time, identify the key signaling and response pathways that are involved in the response of urothelial cells to infection, and learn how to manipulate and interpret large-scale genomic data. These data will form an essential element in either an R01 or a program project and will permit the PI to develop a new line of urologic research.

Grant: 1R21DK063234-01
Program Director: MULLINS, CHRISTOPHER V.
Principal Investigator: BJORLING, DALE E DVM
Title: Role of CD14 and TLR4 in Urothelial Response to LPS.
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 2003/08/10-2005/06/30

DESCRIPTION (provided by applicant): Urinary tract infections result in approximately 9.6 million doctor visits annually, and 20% of women will develop a urinary tract infection during their lifetime. A majority (70-90%) of urinary tract infections are the result of gram-negative bacteria that produce lipopolysaccharide (LPS), and LPS is the component of the cell wall of gram-negative bacteria responsible for cellular response resulting in pain and inflammation. The mucosa of a variety of organ systems (GI, respiratory, urinary) functions as the first line of innate defense against infection by gram-negative bacteria; however, cellular processes that regulate the response of urothelial cells to LPS remain unknown. Although other investigators have reported that urothelial cells do not express CD14 (the primary cognate cellular receptor for LPS), a recent report described the presence of message for CD14 in human bladder mucosa, and preliminary investigations in our laboratory demonstrated the presence of message and protein in human urothelial cells. These cells also express Toll-Like Receptor 4 (TLR4), which appears to provide the transmembrane pathway for signaling in response to LPS. Urothelial cells have the capacity to produce cytokines which recruit and activate leukocytes. The goal of this research is to test the hypothesis that CD14 and TLR4 play a crucial role in the response of the urothelium to LPS. Using cultures of human urothelial cells and mice with genetic disruption of either CD14 or TLR4, we will investigate the response of the urothelium in vitro and in vivo to LPS in the presence or absence of functional CD14 and TLR4. Further, we will determine whether or not the soluble form of CD14 amplifies the response of urothelial cells to LPS. The proposed experiments will better define participation of these receptors in the response of urothelial cells to LPS in the presence (in vivo) and absence (in vitro) of other components of innate immunity. We anticipate that these studies will provide the foundation for continued investigations to identify additional proteins that may participate in this process and dissect signaling pathways which translate LPS binding by urothelial cells into cellular response. The long-range goal of this research is to identify strategies to minimize pain and harmful cellular effects associated with cystitis due to LPS-producing gram-negative bacteria.

Grant: 1R21DK064968-01
Program Director: NYBERG, LEROY M.
Principal Investigator: MOBLEY, HARRY T PHD MICROBIOLOGY, OTTUMWA
Title: Development of a Urea Biosensor Using the UreR Protein
Institution: UNIVERSITY OF MARYLAND BALTIMORE PROF SCHOOL BALTIMORE, MD
Project Period: 2003/07/01-2004/06/30

DESCRIPTION (provided by applicant): Individuals in the U.S. with kidney conditions including infection, kidney stones, cancer, or missing kidney exceed 2.5 million annually. Most renal diseases affect serum urea concentration which provides a sensitive indicator of such disease. The blood urea nitrogen (BUN) test, a routine but critical clinical laboratory assay used to evaluate renal function, quantifies serum urea that is produced in the liver as an endpoint of protein degradation. Studies are proposed that will lead to the development of a fluorescence-based biosensor capable of quantifying urea in a sample in real time. The sensor will employ the UreR protein encoded by the urease operon of the uropathogenic bacterial species *Proteus mirabilis*. UreR, to date, is the only documented urea-binding protein. When UreR binds urea, the protein undergoes a conformational change that is detectable using fluorescence technology. Three approaches to biosensor design will include fluorescent labeling of genetically modified UreR, fluorescence resonance energy transfer of UreR-Yellow-fluorescent-protein fusions, and an anisotropy approach using the binding of its target DNA by UreR. The proposed UreR biosensor will directly measure urea in real time without the necessity of hydrolyzing urea with urease, which is required in current sensor technology. Primary applications may include routine blood urea nitrogen determination and monitoring of blood urea nitrogen directly in the blood of patients undergoing kidney dialysis. In addition, the biosensor will be useful in basic science investigations including urea transport in the liver and kidney, the role of urea hydrolysis in urolithiasis, and urea metabolism in the gastric mucosa infected by urease-positive *Helicobacter pylori*. This biosensor also offers the prospect of imaging urea concentration and fluxes in tissue using fluorescence and confocal microscopy. Our experimental approaches are organized into three Specific Aims: 1) To define the urea binding domain of the UreR protein 2) to develop a urea sensor using UreR; and 3) to construct a prototype UreR-based urea sensor and validate it in specific applications.

Grant: 1R21DK065069-01
Program Director: KARP, ROBERT W
Principal Investigator: KALMAN, DANIEL BA
Title: A C. elegans model for EPEC and EHEC pathogenesis
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 2003/09/15-2005/08/31

DESCRIPTION (provided by applicant): Worldwide, enteropathogenic E. coli (EPEC) and enterohemorrhagic E. coli (EHEC) are lethal human pathogens and are classified as Category B by NIAID. Studies on EPEC and EHEC pathogenesis are limited by an extremely complex genome, comprising 1387 gains and 528 losses compared to E. coli K12, and by a lack of functional assays for many of the proposed virulence factors. We have developed a model for EPEC and EHEC virulence using the nematode C. elegans, which will define novel virulence genes and host targets required for mammalian pathogenesis. Aim 1. EPEC and EHEC strains kill C. elegans under certain conditions, whereas commensal E. coli strains (K12) do not. Killing depends on genes in the LEE pathogenicity island, which are required for EPEC and EHEC pathogenesis in humans. By expressing the LEE in E. coli K12, we will determine whether genes in the LEE are sufficient to cause worm killing, or whether additional genes are required. Aim 2. The tryptophan metabolite indole is secreted and sensed extracellularly by E. coli and regulates stationary phase gene expression. Tryptophan is required for EPEC and EHEC to kill C. elegans. We will determine (i) whether the factor(s) that cause worm killing are secreted by EPEC or require ingestion by the worms; (ii) whether indole contributes directly or indirectly to C. elegans killing; and (iii) whether indole regulates the expression of EPEC and EHEC virulence factors. Aim 3. We will screen large numbers of mutant C. elegans screen for homozygous mutations that confer resistance to killing by EPEC and EHEC. After placement into complementation groups, the precise genes involved will be identified. We will also screen for EPEC and EHEC mutants that fail to kill C. elegans. Together, these studies should yield important information about mechanisms of virulence for these important human pathogens that has not been accessible by traditional experimental approaches. We anticipate that the data generated during the proposed funding period will serve as the basis for an R01 application.

Grant: 1R21DK066630-01
Program Director: SPAIN, LISA M.
Principal Investigator: FASANO, ALESSIO MD
Title: Gut permeability in the pathogenesis of Type 1 diabetes
Institution: UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD
SCHOOL
Project Period: 2003/09/30-2005/08/31

DESCRIPTION (provided by applicant): The trigger of the autoimmune destruction of pancreatic beta cells in Type 1 diabetes is unclear. Several studies suggest that an increased intestinal permeability due to alteration of intestinal tight junctions (tj) can be involved in the pathogenesis of autoimmune diseases, including Type 1 diabetes. The intestinal epithelium is the largest mucosal surface providing an interface between the external environment and the mammalian host. Therefore, it is not surprising that when the integrity of the tj system is compromised an immune response to environmental antigens, including autoimmune diseases, may develop. For the past 10 years our studies have focused on the mechanism(s) of action of a protein elaborated by *Vibrio cholerae*, zonula occludens toxin (Zot) that affects the competency of intestinal tj (3,4). Our experiments using Zot as a tool to gain insights into the regulation of tj function led to the discovery of zonulin, a human eukaryotic Zot analogue, and to the definition of some of its physiological and pathological roles. We extended our findings to disease states, including Type 1 diabetes, characterized by a leaky gut and established the role of zonulin in their pathogenesis. We have applied genetic, biochemical, and biological techniques to establish the structural and functional requirements to activate the zonulin system. Our overall hypothesis is that a subgroup of subjects with Type 1 diabetes experiences a zonulin-dependent abnormal increase in intestinal permeability that causes non-self antigens to cross the intestinal barrier, so triggering an aberrant autoimmune response targeting the pancreatic beta cells. We have assembled a strong interdisciplinary team in which expertise in intestinal tj pathophysiology, cellular and molecular biology, pediatric gastroenterology and endocrinology, diabetology, biostatistics, and human genetics are blend together to offer the best resources to successfully address this hypothesis. The long-term objective of this proposal is to investigate possible links between zonulin-dependent chronic increase in intestinal permeability and the onset of Type 1 diabetes in order to develop strategies for the prevention of the disease. We will use the combination of an animal model of Type 1 diabetes and human studies in order to gain insights into Type 1 diabetes-associated tj dysregulation at the cellular and molecular levels (R21 component of the application). This phase will be followed by human studies performed in our GCRC aimed at developing strategies for the prevention of Type 1 diabetes (R33 component of the application), provided that our milestones will be achieved (Milestones component of the application).

Grant: 1R01EB002175-01
Program Director: MCLAUGHLIN, ALAN
Principal Investigator: HERZFELD, JUDITH PHD CHEMISTRY:PHYSICAL
Title: Structure and Interactions of Gas Vesicles by SSNMR
Institution: BRANDEIS UNIVERSITY WALTHAM, MA
Project Period: 2003/05/01-2008/02/28

DESCRIPTION (provided by applicant): Despite great interest in their remarkable properties, and the considerable range of expertise brought to bear by several groups over many years, only the most general features of the structure of gas vesicles are known. However, recent developments now poise solid state NMR to provide numerous unique probes of the atomic details of the structure of the 7kDa monomer, gvpA, and its assembly into rigid, amyloid-like shells. Preliminary ¹³C and ¹⁵N spectra of uniformly labeled vesicles indicate that a combination of several isotope labeling strategies and an array of multidimensional correlation experiments should allow the full assignment of resonances and the determination of both intra-monomer and inter-monomer structural constraints. Ultimately, this information will allow the complete structure of the gas vesicle shell to be determined. Work will be carried out simultaneously on gas vesicles from the archae Halobacterium salinarum and the cyanobacterium Anabaena flos-aquaea. The parallel efforts will be synergistic because of the strong core homology between the gvpA's of the two species and the different isotope labeling strategies available in the two organisms. The parallel efforts will also be complimentary because of differences in overall vesicle morphology associated with differences in the N- and C-terminal domains of the two gvpA sequences. The role of electrostatics will be explored by identifying the cation displacements and protonation changes that induce vesicle collapse with decreasing pH. The response to salt will also be interesting since H. salinarum is a halophile and A. flos-aquaea is adapted to sweet water. Discovered structural details will shed light on the molecular basis of the morphology and rigidity of the vesicles, the prevention of water condensation within these gas permeable organelles, and the controlled assembly and disassembly of these amyloid-like aggregates.

Grant: 1R01EB002771-01
Program Director: MOY, PETER
Principal Investigator: EDDINGTON, NATALIE PHD PHARMACEUTICS
Title: Delivery of Agents by Modulating Junctions with Zot
Institution: UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD
SCHOOL
Project Period: 2003/09/19-2007/08/31

DESCRIPTION (provided by applicant): The identification of potent macromolecules is increasing at a staggering rate, however many are not delivered effectively due to physiochemical limitations. A viable approach to drug delivery may be to exploit the physiological regulation of intercellular tight junctions, to enhance paracellular drug transport. We have recently demonstrated that Zonula Occludens Toxin (Zot) Zot, a 45kDa protein, exerts its permeating effect by mimicking a eukaryotic analogue in charge of modulation of intercellular tight junctions. This technology allows for enhanced paracellular flux and has the potential to effectively deliver low bioavailable therapeutic macromolecules. Thus our hypothesis is that the Zot technology can enhance drug delivery by reversibly opening tight cellular junctions via oral, BBB and nasal delivery. The following specific aims will be pursued. SA1. To define the structure-function relationships between the major domains of Zot and regulation of the transepithelial/endothelial paracellular pathway. SA2. To determine the dose response, pharmacokinetics (PK) and acute toxicity of Zot. We will define the complete dose-response profile of the Zot (or derivative) in vivo. SA3. To examine the ability of Zot to enhance the oral bioavailability of the hydrophilic agents (mannitol, inulin) and therapeutic macromolecules (insulin, cyclosporin A, paclitaxel). Preliminary data displays that Zot enhances oral absorption of therapeutic molecules. SA4. To examine the ability of Zot to enhance the BBB delivery of the minimally transported CNS therapeutic macromolecules. Zonulin, the ligand for the Zot receptor has been found in the brain and data has shown that Zot enhances the BBB delivery of therapeutic agents (doxorubicin, inulin, acyclovir). SA5. To examine the ability of Zot to act as a novel mucosal adjuvant that enhances the systemic bioavailability of large molecular weight therapeutic macromolecules (e.g., peptides, Protective antigen to B. anthracis) after nasal delivery. Zot has been found to significantly enhance the production of tetanus toxin (TT) antibodies after nasal delivery. The in vivo studies proposed for SA 3-5 will evaluate the ability of Zot to enhance the systemic levels of structurally diverse therapeutic macromolecules. The potential impact of the Zot technology is significant and comprehensive, since it may be applied to delivering a diversified range of macromolecules to their targets via oral, BBB or nasal delivery.

Grant: 2R01ES010182-04A1
Program Director: KIRSHNER, ANNETTE G
Principal Investigator: COFFIELD, JULIE A PHD
Title: Neuromuscular Targets of Botulinum Toxin
Institution: UNIVERSITY OF GEORGIA ATHENS, GA
Project Period: 2000/03/01-2007/07/31

DESCRIPTION (provided by applicant): Botulinum toxin targets the neuromuscular junction (NMJ) producing the fatal paralytic disease botulism. Environmental exposure occurs primarily from contaminated foodstuffs, or from contaminated soil. Exposure to botulinum toxin can also occur through inhalation of aerosolized toxin used as a biological weapon. There are no effective treatment measures for botulism once symptoms appear, and death occurs due to respiratory muscle paralysis. Ironically, botulinum toxin is also a valued drug used to treat neuromuscular diseases characterized by muscle spasticity. Our long range goal is to understand how this toxin selectively targets cholinergic nerve terminals of NMJs, in order to reduce its risk to human health and improve its clinical utility. The objective of this particular application is to define the molecular targets on the nerve terminal membrane that are responsible for the selective action of botulinum toxin serotypes A, B and E at the mammalian NMJ. Substantial evidence indicates that productive binding of botulinum toxins requires both polysialogangliosides and glycoproteins. A number of studies have confirmed the role of G1b gangliosides in toxin binding. The search for the identity of the protein receptors has been far less conclusive. Recently, the nerve terminal proteins synaptotagmin I and II have been proposed as receptors for serotypes A, B and E. However, functional studies in a mammalian NMJ preparation have not been done. To more fully resolve the biochemical interactions of botulinum toxin at its target site, we propose the following specific aims. 1) examine biochemically and functionally the interactions of serotypes A, B, and E with synaptotagmins I and II at the NMJ; 2) determine the identity of NMJ proteins other than synaptotagmins I and II that bind serotypes A, B and/or E. The results of these studies will impact clinical medicine by defining the membrane targets at the NMJ that may serve as templates for the development of effective pharmacologic countermeasures to botulinum intoxication and safer toxin-like therapeutics.

Grant: 1R01ES011903-01A1
Program Director: TINKLE, SALLY S.
Principal Investigator: KOBZIK, LESTER
Title: Inhaled Particles and Host Defense in the Primed Lung
Institution: HARVARD UNIVERSITY (SCH OF PUBLIC BOSTON, MA
HLTH)
Project Period: 2003/07/07-2008/05/31

DESCRIPTION (provided by applicant): The Problem: Hospital admissions for pneumonia are increased by elevated air particle levels. The mechanism(s) underlying particle effects on lung infection are unknown, but may reflect increased incidence of infection, increased severity of infection, or both. Hypothesis: The pathogenesis of the pneumococcal pneumonia (the most common variety and the disease we will study) suggests three possible mechanisms for particle effects: enhancement of lung cell 'receptors' used by bacteria for initial adhesion, damage to antimicrobial function of host cells (AMs and PMNs), and exaggerated inflammation in established infection leading to worse signs and symptoms. Hence, the central thesis of this research is that oxidant components of air particles mediate 1) dysfunction of host defenses against infection (incidence) and 2) increased inflammation in extant pneumonia (severity). Experimental Plan: Aim 1 will measure expression and function of pneumococcal 'receptors' (e.g., PAF receptor) used by pneumococcal for initial adhesion after exposure to concentrated ambient particles (CAPs) or control particles. Aim 2 will determine effects of air particles on pulmonary inflammation before and after onset of pneumococcal pneumonia. The hypothesis to be tested is that particles cause enhanced release of cytokine mediators by primed AMs, leading to increased inflammation and ultimately oxidant damage to both AM and PMN. In vivo and in vitro studies will measure release of pro-inflammatory cytokines, cell influx and viability and severity of pneumonic inflammation. Aim 3 will test the hypothesis that particle exposure inhibits bacterial clearance via oxidant-dependent damage of anti-microbial functions of AMs and PMNs. Component analysis will be performed using a panel of CAPs samples to provide links of particle constituents (e.g., metals, organics, endotoxin) with biologic effects. Rotated factor analysis will be used to correlate source types with CAPs toxicity. Specific intracellular oxidant pathways will be identified by measurement of oxidant production, intracellular levels of antioxidants, and the effect of a panel of anti-oxidants and other inhibitors. Significance: This research is relevant to the public health question of how inhaled particles cause pulmonary health effects and to the pathophysiology of lung host defense against environmental agents.

Grant: 1R21ES012307-01
Program Director: LAWLER, CINDY P
Principal Investigator: CARVEY, PAUL M
Title: Prenatal LPS-induced changes in gene expression
Institution: RUSH UNIVERSITY MEDICAL CENTER CHICAGO, IL
Project Period: 2003/07/01-2006/05/31

DESCRIPTION (provided by applicant): We previously demonstrated that pups born to gravid females treated with the bacteriotoxin lipopolysaccharide (LPS) were born with fewer than normal dopamine (DA) neurons. We have extended these studies out through 16 months and showed that rats exposed to LPS prenatally exhibit progressive loss of DA neurons (46%) associated with increased DA activity, elevations in the proinflammatory cytokine tumor necrosis factor (TNF α), aggregates of alpha-synuclein, and Lewy-like bodies. We have recently received funding from NINDS to characterize this new animal model of Parkinson's disease (PD) through 22 months. However, the mechanism(s) responsible for this DA neuron loss during development are currently unknown. Recent preliminary data (Real-time RT-PCR) suggests that the ratio of pro-/anti-inflammatory cytokine mRNA is increased. In specific aim 1 we will therefore assess both mRNA and cytokine protein (ELISA) content for TNF- α , interleukin (IL) - 113, IL-6, transforming growth factor (TGF β), and IL-6 in the mesencephalon, striatum, and cerebellum (control region) to determine if prenatal LPS alters transcriptional and translational control of these factors during development or early postnatal life (P 1-21). Since Nurr-1, sonic hedgehog, Ptx-3, fibroblast growth factor-8 (FGF8) and glial cell line derived neurotrophic factor (GDNF) are transcriptional and neurotrophic factors, respectively, that regulate the development of the DA neuron phenotype, we will assess mRNA and protein levels of these factors in Specific Aim 2 to determine their involvement in the LPS-induced reduction in DA neurons. Our preliminary data further suggests that LPS induces increases in TNF- α in microglia and we will assess the specific roles of both microglia and astrocytes in the LPS effect in Aim 3. The results of these studies will not only compliment our long-term studies, but will test the hypothesis that prenatal LPS permanently alters genes that regulate the development of the DA neuron and affect its phenotype during adult life. The results of these studies will also provide a unique opportunity to determine if prenatal neurotoxin exposure can permanently alter genes that increase the risk of neurodegenerative disease in later life.

Grant: 2R01EY010974-07A1
Program Director: SHEN, GRACE L
Principal Investigator: O'CALLAGHAN, RICHARD J PHD
Title: Staph Keratitis: Mechanism & Arresting of Corneal Damage
Institution: LOUISIANA STATE UNIV HSC NEW ORLEANS, LA
ORLEANS
Project Period: 1996/08/01-2006/11/30

DESCRIPTION (provided by applicant): Staphylococcus aureus is a leading cause of bacterial keratitis in the United States. Despite prompt and effective antibiotic therapy, this infection can cause corneal scarring that results in blindness or significant loss in visual acuity. No known drug or immune augmentation can prevent these harmful reactions. The long-term goal of this research is to develop therapies to arrest corneal damage and rapidly kill infecting bacteria. Important to contemporary ocular research on Staphylococcus keratitis are findings on the host defense against infection and toxins responsible for tissue damage. The prime host defense in the tear film that protects against corneal infection by Staphylococcus has been identified as phospholipase A2 (PLA2) and inhibition of this bactericidal enzyme has allowed topical infection of the rabbit cornea. Because PLA2 provides a potent innate resistance to infection, lapses in its activity could permit the initiation of infection. Changes in PLA2 activity in response to normal physiologic conditions or to infection, as well as the ability of bacteria to become resistant to this host defense, are important issues in understanding bacterial keratitis that are addressed in Aim 1 of this proposal. The new information on corneal toxins reveals a role for gamma-toxin in corneal damage, a possible synergy between alpha- and gamma-toxins and the activity of an uncharacterized toxin. Aim 2 focuses on the characterization of gamma-toxin, the possible synergy between gamma- and alpha-toxins, and the protectiveness of the immune response to gamma-toxin. Mediating corneal virulence also is a third toxin, one that has yet to be characterized. Aim 3 describes plans to characterize this newly recognized toxin with a focus on its role in virulence, the sequence of the gene for the toxin, and the protectiveness of the immune response to this toxin. The goal of Aim 4 is to clone these toxin genes, in either an active or mutated toxoid form, into a non-pathogenic Staphylococcus species. Such bacteria will be used in the cornea to express the single cloned S. aureus product, thus revealing either its specific toxicity or the immune response to its production in the cornea. These experiments are designed to provide both new knowledge and reagents to help arrest the pathology of S. aureus keratitis.

Grant: 1R01EY014362-01
Program Director: SHEN, GRACE L
Principal Investigator: PEARLMAN, ERIC PHD
Title: Toll like receptors in bacterial keratitis
Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH
Project Period: 2003/04/01-2008/03/31

DESCRIPTION (provided by applicant): Bacterial keratitis is a leading cause of blindness and visual impairment in the developing and industrialized world. Trauma, ocular surface disorders, inappropriate contact lens wear and suture abscess post corneal surgery are among the causative factors that lead to breakdown of corneal defense mechanisms and facilitate access of bacteria and bacterial products into the deeper epithelial and stromal layers. Bacterial products such as lipopolysaccharide (LPS), peptidoglycan (PGN) and unmethylated bacterial DNA (CpG DNA) then activate specific Toll-like receptors (TLR) on resident corneal cells. TLRs initiate a cascade of intracellular signaling events resulting in NFkB translocation into the nucleus, and transcription of proinflammatory and chemotactic cytokines. LPS from most Gram negative bacteria activate TLR4, PGN from gram positive bacterial activate TLR2, and CpG DNA activates TLR9. Activation of specific TLRs induces distinct patterns of cytokine production, despite many shared characteristics of TLR signaling. Experiments outlined in this proposal will utilize TLR and MyD88 deficient mice to identify shared and distinct effects of LPS, PGN and CpG DNA on cytokine and chemokine production by resident corneal epithelial cells and keratocytes, on expression of vascular cell adhesion molecules on limbal vessels and on infiltrating neutrophils and macrophages. Proposed experiments will utilize bone marrow chimeras to delineate the role of TLRs on infiltrating cells from that of resident corneal cells in mediating bacterial keratitis, which will be measured quantitatively by in vivo scanning confocal microscopy. We anticipate that results of these studies will elucidate how signals from TLRs and cytokines integrate in regulating the development and severity of bacterial keratitis, and will lead to the rational design of novel therapeutic agents.

Grant: 1R01EY014847-01
Program Director: FISHER, RICHARD S
Principal Investigator: ARGUESO, PABLO PHD
Title: O-Glycans on Mucins at the Ocular Surface
Institution: SCHEPENS EYE RESEARCH INSTITUTE BOSTON, MA
Project Period: 2003/09/01-2008/08/31

DESCRIPTION (provided by applicant): Mucins are a family of large and heavily O-glycosylated glycoproteins synthesized by all wet-surfaced epithelia, including the corneal and conjunctival epithelia. The ocular surface epithelia produce the secreted mucin MUC5AC and the membrane-associated mucins MUC's 1, 4 and 16. Terminal carbohydrates on mucins are the most exposed to the extracellular milieu, but little is known about their roles and character in each mucin at the ocular surface. Our previous studies have demonstrated that binding of an antibody specific to a terminal carbohydrate on MUC16 is altered in dry eye patients. This proposal is aimed at identifying terminal carbohydrate structures on individual ocular mucins, the enzymes involved in their biosynthesis and the role of terminal O-glycans in maintaining a wet ocular surface and preventing pathogen invasion. **AIM I:** We hypothesize that individual mucins are differentially O-glycosylated by the ocular surface epithelia. We propose to: A. Characterize the repertoire of terminal O-linked carbohydrates on individual mucins isolated from tears of normal individuals. B. Identify and localize glycosyltransferases (sialyltransferases) responsible for their biosynthesis. C. Test in vitro the role of terminal O-linked carbohydrates on mucins of the glycocalyx in conferring disadhesive properties to epithelial cells and resistance to transfection with adeno-associated viruses. **AIM II:** We hypothesize that in dry eye patients there is an alteration in terminal O-glycan structures of the membrane-associated mucins that affects hydration of the ocular surface. We propose to: A. Determine if there is an alteration of terminal carbohydrates of each membrane-associated mucin purified from the apical tear surface of dry eye patients. B. Determine if there is an alteration in the expression of glycosyltransferases (sialyltransferases) in dry eye patients. C. Determine in vitro whether the depletion of terminal carbohydrates on mucins in corneal and conjunctival cells enhances the penetrance of rose bengal into these cells. **AIM III:** We hypothesize that O-linked carbohydrates present on the secreted mucin MUC5AC in the tear fluid have specific affinity to *P. aeruginosa*, preventing their attachment to the ocular surface glycocalyx and facilitating their clearance. We propose to: A. Compare the binding of *P. aeruginosa* to the O-linked carbohydrates of individual mucins collected from tears. B. Determine in vitro how inhibition of O-glycan synthesis on membrane-associated mucins affects *P. aeruginosa* adherence to corneal cells.

Grant: 1R03EY014146-01A1
Program Director: SHEN, GRACE L
Principal Investigator: BONNAH, ROBERT A MOTH
Title: Alteration of host iron homeostasis by Neisseria
Institution: OREGON HEALTH & SCIENCE UNIVERSITY BEAVERTON, OR
Project Period: 2003/09/01-2006/08/31

DESCRIPTION (provided by applicant): *Neisseria gonorrhoeae* (GC) infects a variety of mucosal epithelial surfaces, including the eye, causing hyperacute conjunctivitis. Untreated, these infections can cause visual impairment and blindness. GC infect only humans due to a tropism for human forms of host cell receptors and a requirement for human transferrin (Tf). Tf is a host iron-binding glycoprotein that shuttles iron from sites of absorption to cells of the body. To acquire essential iron in vivo GC produce specific Tf-binding proteins (Tbps) in their outer membrane that allow the piracy of iron from host Tf. GC mutants devoid of Tbps are unable to initiate urethral infection in male volunteers. The goal of the proposed study is to improve current understanding of how pathogenic microbes alter the physiology of cells of the ocular surface, to attain sufficient iron for growth. GC likely play an active role in enhancing their supply of iron on the mucosal surface, by manipulating host conjunctival epithelial cells to downregulate transferrin receptors (TfR), and altering their TfR cycling and TfR distribution patterns. Since animal models for GC eye infections are lacking, conjunctival cell lines and where appropriate, donor tissues will be used to assess uninfected and infected conjunctival cells for downregulated Tf-receptor (TfR) by semi-quantitative RT-PCR. Using a functional assay with radiolabeled Tf and live cells, the levels of host cell TfR will be measured and the TfR cycling rate will be determined in the presence and absence of infection. The other key host iron homeostasis proteins, ferritin, iron regulatory protein-1 (IRP1) and IRP2 levels and biological activity will also be monitored during the course of GC infection. Wild type and isogenic GC mutants lacking specific virulence determinants will be used for the assays. In addition, a specially designed DNA microarray, the 'iron chip', will be used for simultaneous comprehensive analysis of GC alteration of host gene regulation of some 200 genes known to play a role in cellular iron homeostasis. These studies will aid in delineating the specific bacterial products and host signaling factors that may significantly contribute to the processes of iron withholding by host epithelial cells. These studies may lead to novel treatment strategies, since withholding iron can arrest bacterial growth.

Grant: 2P01GM057890-06
Program Director: LI, JERRY
Principal Investigator: MOULT, JOHN PHD BIOPHYSICS, OTHER
Title: From Genomic Sequences to Protein Structure and Function
Institution: UNIVERSITY OF MD BIOTECHNOLOGY BALTIMORE, MD
INSTITUTE
Project Period: 1998/08/01-2008/07/31

DESCRIPTION (provided by applicant): This program project addresses fundamental questions concerning the relationship between genome sequence, protein structure, and function. The present period of the project has focused on structural studies of 'hypothetical' proteins from bacteria. We will now address one of the most surprising outcomes from genome scale studies of higher Eukaryotes: in the last two years it has become clear that in excess of 30% of human genes are expressed in at least one alternatively spliced form. Our thesis is that alternative splicing represents a fundamentally different mechanism for the generation of functional diversity in higher Eukaryotes, but that its utility is moderated by the constraints of maintaining structural integrity. On the one hand, reuse of exons has advantages similar to the reuse of software modules - common sub-function is preserved. On the other, protein substructures are not inherently modular - random combinations of exons would only very rarely lead to viable, folded structures. We aim to thoroughly explore the interplay between these two principles - what kinds of structural change can be accommodated, and what kinds of functional diversity result? In addition to providing an understanding of the relationship between alternative splicing, structure and function, the study will illuminate aspects of protein evolution - under what circumstances can substructures be recombined to generate modified folds? What kinds of conformational change accompany such recombination? Do frame shifts result in folded, completely different structures? Finally, many diseases are associated with alternative splicing. Are the corresponding structures usually non-viable structurally, or do they form alternative structures with pathological functions? To answer these questions, we will determine and analyze a series of carefully chosen structures (50 to 100 over 5 years). Proteins for study will be chosen on the basis of three considerations: likely impact of the splice forms on protein structure, functional role of the splicing, and relevance to disease.

Grant: 2R01GM010452-39
Program Director: ECKSTRAND, IRENE A.
Principal Investigator: KARLIN, SAMUEL PHD
Title: Gene Expression Levels Across Diverse Genomes
Institution: STANFORD UNIVERSITY STANFORD, CA
Project Period: 1979/01/01-2007/04/30

DESCRIPTION (provided by applicant): Prokaryotic and eukaryotic whole genome sequence data is accumulating at an unprecedented pace. The next phase will be increasingly dominated by efforts to characterize, categorize, and analyze these data with the goal of understanding molecular sequence information and its significance in biological systems. Much current biological and medical research centers on DNA microarrays. The main focus of our research is to evaluate gene expression levels based on codon usage. Our sequence methods are complementary to the experimental procedures of 2D-gel electrophoresis in assessing gene expression levels. We have introduced a theoretical computational method for characterizing gene expression levels based on codon usage differences between gene classes. The method has been applied to a variety of genomes including fast-growing bacteria, the cyanobacterium of *Synechocystis* PCC6803, and the radiation resistant *Deinococcus radiodurans* (see Progress Report). We can predict highly expressed genes in each bacterial genome, which correlate very well with 2D-gel protein abundances. We propose to apply the methods to all complete genomes and illustrate here pilot studies for two groups of bacterial genomes: the first group consists of all available low G+C Gram-positive genomes including the pathogens *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and the nonpathogenic dairy fermentation bacterium *Lactococcus lactis*. The second group consists of all available high G+C α -proteobacteria. The latter genomes are important for understanding nitrogen fixation. A second aspect of our research will be to investigate the status of genes in several metabolic pathways and of several protein families among archaeal and bacterial species contrasting presence, absence, and expression levels of genes. A third major objective of our research will be to extend our codon usage methods for predicting gene expression levels to eukaryotic genomes, including yeast, *D. melanogaster*, *C. elegans*, and human.

Grant: 2R01GM013306-38
Program Director: IKEDA, RICHARD A.
Principal Investigator: BENKOVIC, STEPHEN J PHD CHEMISTRY:ORGANIC
SYNTHESIS
Title: Phosphate Activation Mechanisms
Institution: PENNSYLVANIA STATE UNIVERSITY-UNIV UNIVERSITY PARK, PA
PARK
Project Period: 1976/01/01-2006/12/31

DESCRIPTION (provided by applicant): The T4 replication system is a model for DNA replication in general. The replication complex (replisome) is derived from eight proteins that constitute the holoenzyme and the primosome subassemblies which along with single strand binding protein acts at a DNA replication fork. The proposal describes a number of experiments to understand in molecular terms the dynamic and structural characteristics of an active replisome. Key objectives include: defining the composition, dynamics of assembly and disassembly of the primosome; and reconstituting the replisome at a replication fork in order to elucidate the kinetics of its movement with respect to both DNA strands as well as the protein-protein interactions within this multiprotein complex. The techniques to be used either independently or in collaboration span a variety of kinetic methods from rapid quench and stopped-flow fluorescent energy transfer to single molecule imaging as well as structural techniques from cross-linking to electron microscopy and X-ray crystallography. These studies will be extended to include DNA substrates containing lesions in order to gain insights into molecular events surrounding an active replisome attempting lesion bypass, an issue of considerable disease relevance.

Grant: 2R01GM019559-30

Program Director: DEATHERAGE, JAMES F.

Principal Investigator: PARKINSON, JOHN S PHD
GENETICS:BIOCHEMICAL/M
LECULAR

Title: Intracellular Signaling by Bacterial Chemoreceptors

Institution: UNIVERSITY OF UTAH SALT LAKE CITY, UT

Project Period: 1991/06/01-2007/04/30

DESCRIPTION (provided by applicant): The chemotactic behavior of *E. coli* provides the best-studied and most tractable system for elucidating the molecular machinery that also underlies more complex bacterial behaviors, such as host invasion and pathogenesis. The long-term objective of this work is to elucidate the molecular signaling mechanisms of the chemoreceptors that mediate chemotactic behavior in *E. coli* -- how these transmembrane molecules detect and amplify minute chemical signals in the cell's environment and how they generate and modulate intracellular signals to control the cell's flagellar motors. Although chemotaxis in *E. coli* is the best understood biological signaling system, the extraordinary detection sensitivity of bacterial chemoreceptors cannot be explained by known molecular mechanisms. Physical clustering of the cell's receptors may provide an important source of signal gain in the system. Recent genetic and crosslinking studies of the serine (Tsr) and aspartate (Tar) receptors of *E. coli* demonstrated that bacterial chemoreceptors may signal collaboratively in teams comprised of trimers of receptor dimers. Moreover, receptor teams can contain chemoreceptors with different detection specificities working together to control shared kinase molecules (CheA). The specific experimental aims of the proposed project address predictions of the receptor team hypothesis and the mechanisms of receptor signaling: (i) The relationship between team formation, cellular clustering, and signaling sensitivity of chemoreceptors will be investigated with Tsr trimer contact mutants, using fluorescent reporters and in vivo crosslinking to monitor macroscopic and molecular interactions between receptor molecules. If clustering contributes to signal gain, receptors that signal well should cluster well, whereas receptors that cluster poorly should signal poorly. (ii) Physical and functional interactions between chemoreceptors of different specificity will be investigated with Tsr mutants that block Tar function (epistasis) or that regain function in the presence of Tar (functional rescue). Tar and Tsr mutants with compensatory signaling defects will be isolated to examine the behavior of mixed receptor teams. (iii) The signaling properties and structural features of receptor teams will be explored with in vitro assays, using mutant receptors to control team composition and covalent dimers of receptor signaling fragments to control team geometry. (iv) Two new experimental tools will be developed to facilitate these studies: a detergent-solubilized system for studying native chemoreceptor signaling complexes; and chemoreceptor molecules that function with covalently joined subunits. These studies will provide important new perspectives on the issue of receptor clustering, a hallmark of biological signaling systems at every complexity level.

Grant: 2R01GM021371-26A2

Program Director: CHIN, JEAN

Principal Investigator: QUIOCHO, FLORANTE A PHD
BIOPHYSICS:BIOPHYSICS-
UNSPEC

Title: ABC Transport Components: Structures and Functions

Institution: BAYLOR COLLEGE OF MEDICINE HOUSTON, TX

Project Period: 1977/12/01-2007/04/30

DESCRIPTION (provided by applicant): The long-range objective of this research program is to study the structure and function of bacterial ATP-binding cassette-type transport systems (ABC transporters) and their protein components. Structural analysis, using X-ray crystallographic technique, will focus on two components, including several binding proteins that confer the solute specificity of the transporter and chemotaxis as well and the homodimer of the ATPase-hydrolyzing subunits located at the cytoplasmic surface of the membrane. The structures of the dipeptide-binding protein with bound dipeptides may provide fruitful leads in the development of antibacterial drugs (including impermeant peptidomimetic drugs) that exploit the broad specificity of the transporter for delivery and bioactivity. Crystallization of an entire ABC transporter in a transition state will be attempted. Using crystallographic, mutagenic, and biochemical techniques, fundamental investigation of the features of protein-ligand recognition will also be pursued. Transport processes perform a vital function in the life of a cell by maintaining a relative constancy of the intracellular environment and regulating the entrance and exit of various substances necessary for metabolic activity. Chemotaxis is important for the survival (and control) of microorganisms since interaction with the environment depends largely on the ability to respond to stimuli. Beside active transport, the ABC transporters are also involved in signal transduction, protein secretion, antigen presentation, drug and antibiotic resistance, bacterial pathogenesis and sporulation. Several human diseases have been traced to ABC proteins, including cystic fibrosis, hyperinsulinemia, and macular dystrophy. Because several of the substrates of the bacterial ABC transporters are also virulence factors, agents that confer antibiotic resistance, or cellular defense factors on pathogenic bacteria are a growing threat to public health.

Grant: 2R01GM027068-24
Program Director: WOLFE, PAUL B.
Principal Investigator: ROTH, JOHN R PHD GENETICS:GENETICS
UNSPEC
Title: Genetic Analysis of Bacterial Chromosome Structure
Institution: UNIVERSITY OF CALIFORNIA DAVIS DAVIS, CA
Project Period: 1979/12/01-2006/11/30

DESCRIPTION (provided by applicant): This project proposes multiple approaches to the general question of the role of recombination in repair, mutagenesis and genetic adaptation of bacteria. Recent results for provided evidence that the phenomenon know as "adaptive mutation" (Cairns) can be explained purely by standard genetic events that occur during selective growth of cells carrying an amplification of the mutational target. The Amplification model proposes a sequence of genetic events occurring within the developing revertant clone - amplification - mutation - segregation - haploid overgrowth. It argues against the widely circulated ideas of stress-regulated mutation (directed or general), stationary phase mutagenesis or mutagenic recombination. We will continue to characterize this system and test detailed hypotheses to explain the remaining questions, which include the following. What are the exact mechanisms by which amplification induces SOS and activates DinB-dependent mutagenesis? Why does selection-stimulated reversion require that the gene under selection (lac) be located on an F'plasmid? Why does non-essential, reversion-associated general mutagenesis require that lac be located on the specific plasmid (F'128)? Why is growth of the amplification clone inhibited after appearance of the lac reversion? Why do some amplification clones reach maturity (full-sized) revertants without ever achieving lac reversion? We will experimentally measure rates for each step proposed by the model try to mathematically describe the process by which the Cairns system completes a long series of genetic steps within on week of growth under selection. The model promises to shed light on the process of gene evolution, adaptation of pathogens to their hosts and multi-mutational origins of cancer. While mechanisms of replication, recombination, and repair are understood in great detail, it is less clear how these systems interact and how they achieve the amazingly low rate of mutation seen in bacteria. We will continue to pursue a set of assays that measure aspects of recombination as it occurs within the chromosome of growing bacteria. These assays do not involve crosses and require endogenous sources of strand ends to initiate recombinational repair. Thus they can be used to identify endogenous metabolic sources of DNA damage and the role of long-range replication in completion of a recombination event.

Grant: 2R01GM028550-24

Program Director: CHIN, JEAN

Principal Investigator: GRINDLEY, NIGEL D PHD
GENETICS:BIOCHEMICAL/M
LECULAR

Title: Structure and Function of DNA Polymerase I of E.coli

Institution: YALE UNIVERSITY NEW HAVEN, CT

Project Period: 1980/05/01-2007/04/30

DESCRIPTION (provided by investigator): The overall goal of this project is a full understanding, at the molecular level, of the reactions catalyzed by DNA polymerases, with particular emphasis on how polymerases ensure substrate specificity and accuracy in copying DNA. The question of polymerase accuracy has important health implications because the errors made by DNA polymerases can result in mutations leading to human disease. Moreover, DNA polymerases are frequently targeted in chemotherapeutic and antiviral strategies, as well as being important in a variety of diagnostic biotechnology applications, so an understanding of their reaction mechanisms is crucial. Experiments are proposed on two model DNA polymerases that have contrasting enzymatic properties: the highly accurate DNA polymerase I (Klenow fragment) of *E. coli*, and the much less accurate Dbh bypass polymerase from the archaeon *S. solfataricus*. Structural data are available for both these enzymes and several close homologues, and serve as the basis for many of the planned experiments. Moreover, because the important features of the polymerase active site and reaction mechanism are conserved throughout the polymerase family, the results obtained with these simple model systems will have much wider relevance. A major priority will be the investigation of noncovalent steps in the polymerase reaction pathway. These include the early steps involved in substrate recognition and rearrangement of the active site into a form poised for chemical catalysis, and the translocation step that must occur to vacate the active site for the next cycle of addition. A variety of fluorescence assays will be used, in combination with rapid single-turnover kinetics, with the goals of identifying the physical processes involved and understanding their roles in the specificity of the reaction. Fidelity mechanisms at the polymerase active site will be explored using mutants of Klenow fragment. Nucleotide analogues will be used to investigate the role of base pair shape and hydrogen-bonding interactions in the specificity of both Klenow fragment and Dbh polymerase. Mechanistic probes developed for Klenow fragment will be applied to studies of Dbh, in order to learn more about the reaction mechanism and the role of active site side chains in this recently discovered error-prone polymerase. This enzyme has a remarkable propensity for frameshifting, which may provide clues as to its *in vivo* function, perhaps indicating an ability to bypass certain types of bulky DNA lesions.

Grant:	2R01GM029798-24	
Program Director:	SHAPIRO, BERT I.	
Principal Investigator:	RANDALL, LINDA L	PHD MICROBIOLOGY:MICROBL BIOCHEMISTRY
Title:	Export of Proteins in Escherichia coli	
Institution:	UNIVERSITY OF MISSOURI COLUMBIA	COLUMBIA, MO
Project Period:	1981/02/01-2007/03/31	

DESCRIPTION (provided by applicant): The objective is to elucidate the mechanism of protein export in Escherichia coli with emphasis on the interactions of the protein components of the pathway. Translocation of specific, newly synthesized polypeptides across biological membranes is a ubiquitous process, which is essential for living cells. Whether the process occurs in eukaryotes or in prokaryotes in almost all cases molecular chaperones are involved. Chaperones are a family of proteins that display the remarkable ability to recognize and bind polypeptides based on the fact that the ligands are in a nonnative state. The investigators aim to further explore the molecular basis of this sequence-independent recognition by studies of interactions between the chaperone SecB and its ligands. The experiments have been designed to incorporate insights obtained from the x-ray crystal structure. A combination of approaches will be applied to locate the binding site for nonnative polypeptides, to define contacts made and to delineate changes in conformation which occur. Conclusions regarding the molecular mechanism will be confirmed in vitro as well as in vivo by using site-directed mutagenesis to introduce specific changes predicted to eliminate binding and loss of the chaperone activity. In addition to participation of molecular chaperones, a theme common to many biological phenomena including protein export is that of conformational switching of active states. The investigators will provide a molecular description of changes in conformation that serve as activational switches by examining interactions among SecB, polypeptide ligands and SecA, in the presence and absence of other components such as nucleotides and membrane vesicles. The applicants will employ a wide range of techniques to move from a general description of changes in conformation to a molecular description of the events involved at the level of organization of the polypeptide backbone and contacts between side chains. The proposed projects provide a balance among a variety of biochemical and biophysical approaches that complement and reinforce one another. Conclusions that are based on work in vitro with purified proteins will be confirmed in vivo. It is from integration of data obtained through diverse approaches that the investigators will learn the most.

Grant: 2R01GM030498-22
Program Director: LEWIS, CATHERINE D.
Principal Investigator: LOHMAN, TIMOTHY M PHD CHEMISTRY:PHYSICA
Title: SSB PROTEIN/DNA INTERACTIONS
Institution: WASHINGTON UNIVERSITY ST. LOUIS, MO
Project Period: 1990/07/01-2007/03/31

DESCRIPTION (provided by applicant): A molecular understanding is sought of the interactions of the Single Stranded DNA Binding (SSB) proteins from *E. coli* the homo-tetrameric EcoSSB, and yeast, the hetero-trimeric RPA; with single stranded (ss) DNA using thermodynamic, kinetic, single molecule and structural approaches. Both of these proteins bind selectively to ssDNA and are essential for DNA replication and repair and facilitate recombination. EcoSSB serves as a paradigm for a growing number of similar proteins from other organisms. EcoSSB displays a complex array of multiple ssDNA binding modes, multiple inter-tetramer positive cooperativities and a negative cooperativity for ssDNA binding within an individual tetramer that regulates the binding modes. These different binding modes, which differ in the extent to which DNA is wrapped around the tetramer, are likely used selectively in different processes in vivo. The EcoSSB protein also serves as a model to understand the fundamental thermodynamic profile of a protein/ssDNA binding system. A major goal is to understand the effects of salt concentration on the thermodynamics and kinetics, since electrostatic effects are a prominent component of protein-nucleic acid systems. The thermodynamics of oligodeoxynucleotide binding will be examined by isothermal titration calorimetry (ITC), to obtain ΔG_{obs} , ΔH_{obs} , ΔS_{obs} and ΔC_P , obs as a function of solution conditions, focussing on the extremely large and favorable ΔH_{obs} and the dramatic dependence of ΔH_{obs} and ΔC_P , obs on salt concentration. The thermodynamics of cooperative ssDNA binding in both the (SSB)₃₅ and (SSB)₆₅ binding modes will be examined and single molecule fluorescence techniques will be used to examine ssDNA wrapping. Kinetic mechanisms of ssDNA binding, ssDNA wrapping and "direct transfer" of SSB between ssDNA molecules will be examined using fluorescence stopped-flow and temperature-jump techniques. The energetics of EcoSSB binding to replication proteins such as the χ subunit of the gamma complex of DNA pol III will be pursued. High resolution structural information will also be sought on complexes of EcoSSB with ssDNA and the X subunit through x-ray crystallographic analysis in order to place the thermodynamic studies in a structural context. Finally, thermodynamic studies of ssDNA binding to the hetero-trimeric RPA protein, the eukaryotic analog of EcoSSB, will be pursued to examine its ability to bind in multiple binding modes, and compare its properties to those of EcoSSB.

Grant: 2R01GM031693-22
Program Director: ANDERSON, RICHARD A.
Principal Investigator: SMITH, GERALD R PHD
Title: Molecular Analysis of Hotspots of Genetic Recombination
Institution: FRED HUTCHINSON CANCER RESEARCH SEATTLE, WA
CENTER
Project Period: 1982/07/01-2006/12/31

DESCRIPTION (provided by applicant): The long-term goal of the proposed research is to elucidate the molecular mechanism of homologous genetic recombination. This goal is approached by studying hotspots of recombination, which stimulate a critical, rate-limiting step of recombination. In the bacterium *Escherichia coli*, studies will focus on Chi hotspots, which stimulate the major (RecBCD) pathway of recombination and DNA break repair. In the fission yeast *Schizosaccharomyces pombe*, studies will focus on the M26 hotspot and the recently discovered hotspot mbsl, both of which stimulate meiotic recombination through the formation of DNA double-strand breaks. A unifying theme emerging from the research in *E. coli* and *S. pombe* is that homologous recombination can frequently occur far from broken DNA ends. The specific aims are 1) to elucidate the complex interaction of Chi hotspots and RecBCD enzyme, with special emphasis on the interdependencies of the multiple enzymatic and physical changes of RecBCD enzyme and DNA resulting from this interaction, 2) to determine the basis of the context-dependence of M26 hotspot activity and to assess the role of M26-like sites in wild-type *S. pombe*, and 3) to investigate the mbsl hotspot, with special emphasis on testing the hypothesis that meiotic gene conversion and crossing-over can be separated by a long distance (tens of kilobases). These aims will be achieved by a combination of biochemistry and electron microscopy with purified components, and genetics with intact cells. The results of these studies will elucidate the regulation of recombination both spatially along chromosomes and temporally during the organism's life cycle. Recombination is important in the faithful repair of DNA double-strand breaks in chromosomes and in the faithful segregation of chromosomes during meiosis. Aberrancies of recombination and DNA break repair are responsible for chromosomal rearrangements associated with cancer, birth defects, and certain hereditary diseases. Gene therapy by homologous gene replacement and gene targeting to generate experimental animals will be facilitated by understanding the molecular mechanism of homologous recombination and its stimulation by hotspots.

Grant: 2R01GM031986-20A1
Program Director: CHIN, JEAN
Principal Investigator: LONDON, ERWIN PHD
Title: Diphtheria Toxin: Structure and Membrane Interaction
Institution: STATE UNIVERSITY NEW YORK STONY BROOK STONY BROOK, NY
Project Period: 1983/04/01-2007/03/31

DESCRIPTION (provided by applicant): This project aims to understand how bacterial proteins penetrate cell membranes and enter the cytoplasm of mammalian cells in order to understand how proteins can cross membranes. The primary subject of study is diphtheria toxin. Its membrane insertion occurs after exposure to the low pH within the lumen of endosomal vacuoles. The A chain of the toxin then translocates into the cytoplasm, aided by the toxin's hydrophobic T domain. To analyze this process, the structure of membrane-inserted A and T subunits will be studied, making use of site-directed mutagenesis combined with in vitro fluorescence-based assays. In addition, function will be evaluated by pore formation, translocation assays and cellular toxicity measurements. The role of the T chain will be analyzed by assessing the effects of blocking the insertion of individual T domain helices on membrane-inserted structure and function. Also, crucial T domain residues will be identified and their effect on structure and function characterized. The translocation mechanism will be studied by examining the role of pore formation, oligomerization, and the covalent link between the A and T chain on translocation. In addition, the role of interactions between the A and T chain in translocation will be analyzed by comparing their topography in the membrane-inserted A-T complex before, after and during translocation. Finally, the hypothesis that the T domain promotes translocation by acting like a relatively non-specific transmembrane chaperone will be tested by assaying the translocation of chimeras with the A chain replaced by proteins that vary in their degree of folding and/or hydrophobicity. Studies will then be extended to the type III translocation system of pathogenic bacteria. Interesting parallels between diphtheria toxin and type III translocation have recently become apparent. Our first target will be the YopB and YopD proteins of Yersinia. They are main components of the membrane-perforating apparatus through which translocating proteins pass. Our first goal will be to understand the relationship of YopB/D function to their topography when in the membrane-inserted state, with a longer-range goal being exploration of how YopB/D interactions with other Yersinia proteins results in the assembly of a functional translocation pore. An ultimate goal is to aid development of therapeutic agents interfering with infection. In a first step, the effects of molecules found to inhibit T domain pore formation upon YopB/D and Yersinia pathogenesis will be tested.

Grant:	2R01GM032506-22	
Program Director:	ZATZ, MARION M.	
Principal Investigator:	SHAPIRO, LUCILLE	PHD MICROBIOLOGY:MICROBIO OGY-UNSPEC
Title:	Regulation of Differentiation in Caulobacter	
Institution:	STANFORD UNIVERSITY	STANFORD, CA
Project Period:	1986/07/01-2007/06/30	

DESCRIPTION (provided by applicant): Our goal is to determine how multiple regulatory mechanisms are linked together to form a system that controls polar morphogenesis and cell cycle progression in *Caulobacter crescentus*. The completion of the annotated genome sequence of 3767 genes, ease of synchrony and genetic manipulation, and full genome microarray analysis have allowed us to explore the global regulatory network that controls a bacterial cell cycle. Temporally regulated transcription, signal transduction mediated by the two component family of proteins, and temporally controlled proteolytic events all contribute to the control of the *Caulobacter* cell cycle. In addition, we discovered that regulatory proteins that control the cell cycle and asymmetry at cell division are dynamically localized to cell poles. We will now determine why and how these proteins are dynamically positioned to specific sites in the cell. Temporally controlled clearance from the cell of the components of the polar pili, flagellum, and chemotaxis complex, and the regulatory proteins that regulate their biogenesis, is critical for the establishment of asymmetry. We will determine the mechanisms that are used to control the proteolysis of both structural and regulatory proteins at critical times in the cell cycle. Finally, we have found that of 550 genes whose transcription is regulated during the cell cycle, 25 percent are controlled by the CtrA response regulator. We will now identify the global regulators that control temporally expressed genes that are independent of CtrA control. We will also identify the groups of genes that are controlled by 5 new regulatory proteins that are themselves directly controlled by CtrA at specific times in the cell cycle in an attempt to construct a serially connected network that integrates the genetic circuitry. The proposed experiments will explore individual regulatory mechanisms in depth while determining how they form a three-dimensional network for the control of the cell cycle.

Grant: 2R01GM033328-19
Program Director: SCHWAB, JOHN M.
Principal Investigator: EVANS, DAVID A PHD CHEMISTRY:ORGANIC
Title: SYNTHESIS OF AMINO ACID-DERIVED NATURAL PRODUCTS
Institution: HARVARD UNIVERSITY CAMBRIDGE, MA
Project Period: 1983/08/01-2007/03/31

DESCRIPTION (provided by applicant): Chemical synthesis provides the capacity to produce chemotherapeutic agents, and chemical reactions are the irreplaceable tools of the medicinal chemist engaged in the drug discovery process. Advances in chemical reaction technology reduce the interval between the conception of the chemical entity as a potential drug candidate and its synthesis for subsequent biological evaluation. As a consequence, the synthesis activity is a critical discipline that continues to have an important impact upon the fields of both medicine and biology. The present grant will continue to address the development of new stereoselective reactions which are relevant to the synthesis of antibiotic and antineoplastic agents derived from amino acid constituents. The methodological studies dealing with new reaction discovery will emphasize the development of chiral metal catalysts for the synthesis of complex amino acids. These advances should culminate in practical routes to the asymmetric synthesis of beta-hydroxy-alpha-amino acids and alpha, beta-diamino acids. Synthesis targets associated with this project will include the antiviral cyclic heptapeptide cyclomarin A, the marine toxin, azaspiracid 1, and the cytotoxic hexacyclic alkaloid daphnycyclidin A.

Grant: 2R01GM035754-18
Program Director: TOMPKINS, LAURIE
Principal Investigator: GRALLA, JAY D PHD
Title: Mechanism of Repression in Bacteria
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 1985/12/01-2007/08/31

DESCRIPTION (provided by applicant): The structure of RNA polymerase is very similar from bacteria to man. This enzyme is responsible for the bulk of differential gene expression in all organisms. The properties of the large enzyme are modified by interactions with proteins and small molecule effectors. In this proposal a bacterial system is used to learn how the common core can have its properties changed. 3 sigma factors are used, the main housekeeping sigma70, sigma38 and sigma54, each of which causes the core to assume a different mechanism of action. Sigma38 causes the core to respond uniquely to several small molecule effectors. Sigma54 causes the core to respond to enhancer binding proteins. Sigma70 induces neither of these responses. A combination of genetic screens, band shift assays and footprinting analysis will be used to investigate this problem. Screens are devised to identify the parts of sigmas that are involved in these responses. The biochemical assays are used on the mutants obtained to learn why they are no longer able to alter the behavior of the core RNA polymerase. These mutants will be mapped on the known high resolution structures. The outcome is expected to be a description of the 3-dimensional domains on sigma that are critical for changing how the core polymerase works and new knowledge about how this happens. Because the core polymerase core is so conserved we expect that its manner of response will also be conserved, enlarging the possibilities for understanding the basis for human diseases of gene expression.

Grant: 2R01GM037048-17
Program Director: TOMPKINS, LAURIE
Principal Investigator: GOURSE, RICHARD L. PHD
Title: Mechanism, Activation, and Control of rRNA Transcription
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 1988/07/01-2007/06/30

DESCRIPTION (provided by applicant): The machinery responsible for making proteins (e.g. ribosomal RNA, ribosomal proteins, translation factors, and tRNAs) is central to growth and development of all organisms. The regulation of the synthesis of the translation machinery in response to the nutritional state of the cell has been a central issue in the study of microbial physiology for at least fifty years. More recently, it has also become clear that an understanding of the mechanisms responsible for rRNA transcription in *Escherichia coli* can provide fundamental insights into understanding mechanisms of transcription in general. We are poised to address questions central to our understanding of general transcription mechanisms and to our understanding of how different regulatory systems work together. The questions addressed in the proposal are divided into four specific aims in arbitrary order. In the first aim, we will study an unanticipated role of the C-terminal domain of the alpha subunit of RNA polymerase in bacterial promoter recognition. In the second aim, we propose to determine how the *rrn* transcription factor Fis increases transcription from sites far upstream of the -35 element and how it affects different steps in the transcription mechanism. In the third aim, we propose to determine when different mechanisms contribute to regulation of the *rrn* P1 and *rrn* P2 promoters in response to changing nutritional conditions and how this maintains homeostasis. Also as part of this aim, we will determine the molecular interactions responsible for a key determinant in the control of rRNA transcription, the lifetime of the open complex, we will examine a new, previously unrecognized regulator of rRNA transcription, and we will begin a genomic study of tRNA promoters. In the fourth specific aim, we will use the tools of cell biology to determine the locations of rRNA operons in the bacterial nucleoid.

Grant: 2R01GM039277-13
Program Director: SOMERS, SCOTT D.
Principal Investigator: VARY, THOMAS C MS CELL BIOL
NEC:MOLECULAR BIOLOG
Title: REGULATION OF PROTEIN TURNOVER IN SEPSIS
Institution: PENNSYLVANIA STATE UNIV HERSHEY HERSHEY, PA
MED CTR
Project Period: 1989/07/01-2007/07/31

DESCRIPTION (provided by applicant): The objective of the studies described herein is directed at defining the biochemical basis for the impairment in protein synthesis that characterizes the metabolic response to sepsis. Sustained loss of skeletal muscle protein contributes to the morbidity and mortality associated with sepsis. By understanding the derangements in the process of protein synthesis it is hoped that new strategies could be developed to combat the severe muscle wasting associated with the septic episode. We have established that two regulatory steps in the process of mRNA translation initiation phase of protein synthesis in skeletal muscle become inhibited during sepsis. These two loci are the reactions catalyzed by eIF2B and the assembly of an active eIF4E.eIF4G complex. We hypothesize that the normal pathways in muscle responsible for maintaining the functioning of these two steps in mRNA translation initiation are severely compromised during sepsis. Once we have clarified the altered regulation of these two steps in sepsis, we will investigate different approaches (e.g. anti-cytokine therapies; amino acids; IGF-I) to circumvent the derangements identified. The Specific Aims for the forthcoming project period are: 1) To define the mechanism(s) by which sepsis increases the phosphorylation of the E-subunit of eIF2BE in skeletal muscle by examining the regulation of glycogen synthase kinase 3; 2) To examine the role of phosphorylation of eIF4G in the control of formation of eIF4E.eIF4G complex in gastrocnemius of septic rats; 3) To determine the role of rapamycin-Insensitive pathway in the control of formation of eIF4E.eIF4G complex during sepsis following provision of amino acids and to delineate the signal transduction pathways regulated by amino acids through cDNA Expression Array analysis; and 4) To examine the mechanism by which IGF-I, but not insulin, decreases phosphorylation of eIF2BE and/or increases formation of the active eIF4E.eIF4G complex in the stimulation of protein synthesis during sepsis. To accomplish these aims we will use a carefully characterized rat model of chronic, intra-abdominal sepsis whereby we can dissociate the effects of sterile inflammation from those of sepsis. The proposed studies should lead to potential new therapies to limit the loss of skeletal muscle protein during sepsis.

Grant: 2R01GM041883-14
Program Director: WEHRLE, JANNA P.
Principal Investigator: BECKWITH, JONATHAN R PHD
Title: Genetics of Bacterial Thiol Redox Proteins
Institution: HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA
Project Period: 1989/06/01-2006/11/30

DESCRIPTION (provided by applicant): The goal of this project is to understand the physiological and molecular basis for the wide variety of important cellular activities of proteins with disulfide bond oxidoreductase activity. We focus on the members of this family of proteins in the bacteria *E. coli*, including the thioredoxin superfamily. We will explore the differing roles of these proteins by 1) studying the regulation of their synthesis in order to define important cellular responses they are involved in, 2) by trapping mixed-disulfide complexes of these proteins with their substrates (this will be done to define heretofore undetected substrates of these proteins), and 3) isolating suppressor mutations in strains missing many of these components. We anticipate that characterization of these suppressor mutations will reveal additional members of the protein family and provide new information on the function of known members. We will also determine how the thioredoxin family, members of which have highly conserved three-dimensional structures, often exhibit such different substrate specificities. Swap constructs and mutations that alter specificity differences between the thioredoxins 1 and 2 will shed light on this question. Selection for altered specificity mutations is based on the failure of thioredoxin 2 to reduce the enzyme methionine sulfoxide reductase. The genetic studies will be combined with structural information obtained in a collaborative effort with an X-ray crystallographer. We will characterize in depth the mechanism of action of the protein DsbB. DsbB is a membrane protein that is required for the reoxidation of the thiol oxidase, DsbA, passing its electrons to quinines. We have dissected this process into several steps. A collection of DsbB mutants already isolated will be used to determine the role of different domains of the protein in this complex series of steps. Structural information on the protein obtained from both a collaborative NMR effort and genetic studies will be combined with this mutant analysis to understand the functioning of this protein. Studies on pathways of disulfide bond formation and reduction have already provided benefits for the enhanced production of medically important proteins such as antibodies and tissue plasminogen activators and this study should provide additional information for such efforts. Furthermore, these proteins play an important role in a host of cellular processes, both normal and pathological.

Grant: 2R01GM042033-14A1
Program Director: SHAPIRO, BERT I.
Principal Investigator: OLIVER, DONALD B PHD
Title: Mechanism of Protein Localization in Escherichia coli
Institution: WESLEYAN UNIVERSITY MIDDLETOWN, CT
Project Period: 1989/04/01-2007/03/31

DESCRIPTION (provided by applicant): Our overall goal is to elucidate the molecular details of protein translocation across biological membranes utilizing Escherichia coli as a facile genetic and biochemical system. We will focus on a central component, SecA ATPase, which interacts with most components of this system, and whose ATPase and membrane integration activities are at the heart of the energetics and mechanism of translocation. Three specific aims are proposed. (1) To understand SecA-translocon interaction and its dynamics sulfhydryl labeling of SecA-Cys proteins in right side-out membrane vesicles will be employed to develop a high resolution membrane topology map of SecA in comparison to its solution structure. A subunit-switching model of SecA action will be tested. (2) To understand the structural basis of signal peptide binding to SecA and its regulation, the interaction of SecA with a labeled signal peptide will be studied and fluorescence resonance energy transfer will be utilized to map this site on the SecA structure. This approach will also be used to follow inter-domain movement in SecA containing appropriate fluorescent labels to test a clamshell model for regulating access to the signal peptide-binding site (3) The secretion-specific regulation of secA by the translational pause in secM will be studied genetically to define the secM translational-arrest peptide and those components of the translation and secretion machineries that regulate pausing. The requirement for translocon "pulling" of nascent translocating SecM as the signal for releasing the secM translational pause will be tested. These studies should lead to a refined picture of SecA structure, biochemistry, mechanism, and its regulation. They should be of broad significance to understand these processes in parallel systems, to engineer such pathways, and to develop novel anti-bacterial agents.

Grant: 2R01GM042146-13
Program Director: SHAPIRO, BERT I.
Principal Investigator: POSTLE, KATHLEEN PHD BIOCHEMISTRY
Title: Energy Transduction Between Membranes
Institution: WASHINGTON STATE UNIVERSITY PULLMAN, WA
Project Period: 1991/08/01-2007/07/31

DESCRIPTION (provided by applicant): Primary energy transduction in biological systems is derived from two coupled sources. Oxidative phosphorylation (and substrate phosphorylation) ultimately generate both an ion motive force and cytoplasmic ATP, in reactions where the reactants and products are physically and spatially coupled. Both of these energy sources are used to drive active transport of nutrients across the cytoplasmic membrane. The outer membranes of Gram-negative bacteria present a special problem: They are unenergized and they are impermeant to molecules of greater than 600 Da. In order to obtain vital nutrients, these organisms have developed a sophisticated system whereby energy generated by conventional means (cytoplasmic membrane protonmotive force) can be transduced to transport proteins in the outer membrane for active transport of nutrients into the periplasmic space. This system appears to constitute a new biological paradigm. Using *E. coli* as a model system, it has become clear that several proteins are involved in this process, at both the cytoplasmic and outer membranes. The central player in this process, TonB, is hypothesized to carry conformationally constrained potential energy, and shuttle back and forth between the two membranes to obtain and then deliver it. In the cytoplasmic membrane ExbB and ExbD are hypothesized to convert Ton B to its energized form and to provide the energy that runs the shuttle. In the outer membrane are proteins with which TonB may dock as well as the outer membrane transporters to which the energy is delivered. In this proposal a further understanding of the molecular details of the energy transduction mechanism are sought. Specifically, we will 1) define the functions of the TonB amino and carboxy termini 2) investigate the energy source for the shuttle 3) continue to define the roles of ExbB and ExbD, and 4) initiate studies, based on the structure of colicin B, to characterize the stages of TonB-dependent colicin infection, starting at the outer membrane.

Grant: 2R01GM042219-14A1
Program Director: ANDERSON, JAMES J.
Principal Investigator: SONENSHEIN, ABRAHAM L PHD
MICROBIOLOGY:MICROBIOLOGY
PHYSIOLOGY
Title: Isolation of Early Sporulation Genes
Institution: TUFTS UNIVERSITY BOSTON BOSTON, MA
Project Period: 1989/07/01-2007/08/31

Bacillus subtilis, an endospore-forming bacterium, is, after *Escherichia coli*, nature's best studied organism and has proved to be a useful model for other gram-positive bacteria, pathogens and non-pathogens alike. Endosporulation in *Bacillus* and *Clostridium* spp. is a response to severe nutrient limitation. Thus, the metabolic signals that the cell perceives are a crucial component of the regulation of this differentiation process. In recent prior work, the *B. subtilis* CodY protein has been identified as a metabolite-sensing repressor that controls a number of genes that are turned on when cells experience nutrient deprivation. Some of these genes must be critical for sporulation, because a *codY* mutant sporulates under conditions of nutrient excess. It has long been recognized that the intracellular concentration of GTP drops when cells make the transition from rapid exponential growth to stationary phase. CodY has now been shown to respond to the intracellular level of GTP, losing activity as a repressor as the GTP concentration drops. CodY also responds to isoleucine and valine, two of the branched chain amino acids. These novel findings suggest that CodY plays a surprisingly general role in cellular metabolism and differentiation. In fact, microarray analysis has shown that hundreds of genes respond to the absence of CodY and are thus either direct or indirect targets of the regulatory protein. This proposal seeks to address several fundamental questions about the role of CodY and the mechanism by which it regulates transcription. The specific aims of the proposal are: (i) to determine the role of CodY in initiation of sporulation; (ii) to determine the global role of CodY in metabolism; (iii) to determine the molecular architecture of CodY and its interaction with effectors; (iv) to characterize the interaction between CodY and its DNA targets; and, (v) to identify a hypothetical second signaling pathway that mediates the switch from stationary phase to sporulation. The results of this research have the potential to resolve longstanding mysteries, such as why the onset of sporulation is accompanied by a transient drop in the GTP pool, and to provide new insight into the metabolic regulation of stationary phase and early sporulation events. Given the existence in other gram-positive bacteria of proteins that are remarkably similar to CodY, it is likely that the findings of this project will have general application to a broad group of important prokaryotes.

Grant: 2R01GM042893-14
Program Director: ANDERSON, JAMES J.
Principal Investigator: WINANS, STEPHEN C
Title: Interactions Between Agrobacterium and Host Plants
Institution: CORNELL UNIVERSITY ITHACA ITHACA, NY
Project Period: 1989/07/01-2007/02/28

DESCRIPTION (provided by applicant): The long-range goal of this lab is to continue developing the Agrobacterium-plant pathosystem as a model for studying molecular interactions between pathogenic bacteria and their hosts. After the successful oncogenic transformation of host plants, *A. tumefaciens* colonizes the transformed plant and uses tumor released compounds (opines) as nutrients. Under these conditions, the bacterium uses a quorum-sensing system composed of *Tral*, which synthesizes the bacterial pheromone N-3-oxooctanoylhomoserine lactone (OOHL) and *TraR*, which is an OOHL receptor and OOHL-dependent transcriptional regulator. We have purified both proteins and reconstituted their activities in vitro. We recently collaborated in solving the crystal structure of *TraR*, complexed with OOHL and DNA. We will take advantage of this structure to do structural studies of *TraR*, by doing alanine-scanning mutagenesis of the *TraR* surface, its OOHL binding determinants, and its DNA binding determinants. We will attempt to isolate positive control mutants, and will do a selection for constitutively active *TraR* mutants and for mutants able to detect heterologous autoinducers. We have shown that *TraR* synthesized in the absence of OOHL is rapidly targeted for proteolysis, probably by the *Cip* and *Lon* proteases. We will disrupt the *Ion* and the three *clpP* genes of *Agrobacterium* and measure the half life of the protein, and whether it still requires OOHL for stability and activity. We will overproduce the N-terminal domain of *TraR* in the presence of C13 and N15 isotopes for collaborative studies of *TraR* folding. We will express the C-terminal domain in vivo in a form that dimerizes to check for DNA binding and for transcription activation. We have been to compare the biochemical properties of *TraR* to those of two other homologous proteins: *CepR* of *Burkholderia cepacia*, and *YenR* of *Yersinia enterocolitica*. *CepR*, requires its cognate pheromone for solubility, while *YenR* does not. In this respect, *YenR* is especially interesting, as we can purify this protein as an apo-protein or as an AHL complex and compare their properties. *YenR* binds to its binding site near the *YenR* promoter only in the apoprotein form, suggesting that the pheromone antagonizes protein function. We will do standard biochemical analysis of *YenR* will use several approaches to identify target genes of the *YenR*-*YenI* regulon. In a final project, we will screen for AHL synthase genes from DNA that is purified from environmental samples and cloned into cosmids in *E. coli*.

Grant: 2R01GM042897-22
Program Director: SCHWAB, JOHN M.
Principal Investigator: WILLIAMS, DAVID R.
Title: Interesting Bioactive Substances
Institution: INDIANA UNIVERSITY BLOOMINGTON BLOOMINGTON, IN
Project Period: 1989/07/01-2007/06/30

DESCRIPTION (provided by applicant): Plans describe the continuation of an ambitious and successful program investigating fundamental chemistry directed towards the syntheses of unique, biologically active natural products. The research plan details innovative solutions and strategies which address the challenging aspects of bond construction and stereochemistry presented by these highly functionalized marine metabolites. The program is organized into two categories. I. Macrolactone Antitumor Antibiotics. Part A: Plans for the synthesis of amphidinolide C are presented. Marine macrolides of the amphidinolide family are among the most potent antitumor agents discovered, with remarkable activity in nearly all NCI tumor cell lines. Extremely limited quantities have hampered complete structural elucidations and biological studies. The proposed chemistry develops new functionalized allylic and allenyl reagents for the efficient and stereocontrolled formation of sensitive, densely functionalized components. Studies include the extension of Pd(0)-coupling reactions in this demanding context to lead to the first synthesis of amphidinolide C. Part B: The powerful antitumor and antifungal properties of leucascandrolide A have generated considerable attention. Our asymmetric allylstannane methodology promises to deliver the most stereoselective approach yet devised for rapid construction of the alternating 1,3,5-oxygenation pattern which characterizes this unique macrolactone. II. Novel Carbocyclic Antibiotics. Part A: Studies are directed toward kendomycin, a potent antitumor quinone methide system, displaying a conformationally restricted 18-membered ansa bridge. Our strategic plan describes key oxidation chemistry of benzofurans. Ring-closing metathesis, Julia condensations, and pinacol couplings will be explored for macrocycle formation. Diels-Alder reactions will examine opportunities for synthesis of complex 1-arylpyrans as a generalized approach toward C-aryl glycosides. Methodology for preparation of contiguous stereotriads will utilize allenylstannanes. Part B: Efforts toward the synthesis of nine-membered, cytotoxic marine xenicanes will investigate strategies for rapid construction of these conformationally constrained carbocycles from functionalized acyclic precursors. A thematic presentation of ring formation strategies and developments for Pd(0) chemistry fuel these efforts. The selective synthesis of contiguous stereotriads will advance basic methodology for asymmetric conjugate additions.

Grant: 2R01GM043756-13

Program Director: WOLFE, PAUL B.

Principal Investigator: DUBNAU, DAVID A

PHD

GENETICS:BIOCHEMICAL/M

LECULAR

Title: Genetic Competence Apparatus of Bacillus Subtilis

Institution: PUBLIC HEALTH RESEARCH INSTITUTE NEW YORK, NY

Project Period: 1990/12/01-2006/12/31

DESCRIPTION (provided by applicant): Natural transformation in bacteria refers to the uptake and integration of exogenous DNA, Bacillus subtilis can be transformed by exogenous DNA when in a physiological state known as competence, Competent cells are able to bind double stranded DNA, fragment the DNA on the cell surface and transport a single strand across the cell membrane, Our long-term objective is to understand these processes on the molecular level. This proposal explores the specific roles of proteins known to participate in the binding on processing and transport (internalization) of transforming DNA, Several of these proteins (the seven comG gene products, ComC and ComEA) are needed for the binding of DNA to the cell surface while others (ComEC, ComFA and ComEA) are required for transport of DNA across the cell membrane. The proposed work will explore interactions among these proteins and the role of the thiol-disulfide oxidoreductases BdbD and BdbC in the folding of these proteins and in the assembly of the DNA uptake apparatus. The formation and properties of a newly identified higher order ComGC complex will be elucidated and its role in DNA binding will be examined. The structure of the putative membrane channel protein ComEC and the in vitro biochemical activities of the transporter ATPase ComFA will be characterized further. The amounts of these various proteins in the cell will be determined, Several newly identified transformation proteins will be studied, Finally an attempt will be made to develop a vesicle-based in vitro DNA transport system.

Grant: 2R01GM046354-11A1
Program Director: SOMERS, SCOTT D.
Principal Investigator: AYALA, ALFRED PHD REGULATORY BIOLO
Title: Differential Effects of Sepsis on Macrophage Function
Institution: RHODE ISLAND HOSPITAL (PROVIDENCE, PROVIDENCE, RI
RI)
Project Period: 1991/09/30-2007/08/31

DESCRIPTION (provided by applicant): The inability of the present therapies to mitigate the devastating effects of sepsis and multiple organ failure in the critically ill patient indicates that more knowledge of the pathophysiology of sepsis is needed if we are to develop newer, more effective interventions. In this respect, our studies, using a model of chronic polymicrobial septic mortality (i.e., cecal ligation [CL] and puncture [CLP]), indicate that it is the response to devitalized/injured tissue present in CLP that appears to predispose the host to the induction of a suppressive lymphoid and/or macrophage (M/phi) phenotype. Microbial stimuli, in turn, serve to induce the subsequent immune dysfunction (pro-inflammatory & Th1-function) as well as the mortality seen in CLP. We have shown that the production of IL-10, IL-4, TGF-beta and/or NO appear to contribute to the suppression of Th1/pro-inflammatory responsiveness seen in late sepsis in a tissue specific fashion via p38 MAPK, STAT-6, and/or SOCS proteins. In the spleen, this appears to be the result of Th2-cell differentiation. Conversely, preliminary studies indicate that gamma8 T-cells and/or CD8+-T-cells play a role in changes in the innate and not the cellular immune response to septic challenge. Alternatively, mice inhibited in their ability to activate an NK-T-cell response exhibit improved septic survival. In light of the above, this proposal will test the hypothesis that the immune hypo responsiveness, thought to predispose these animals to multi-organ failure and death as seen in sepsis, is the result of a dysfunctional response to infectious challenge in the presence of necrotic /injured tissue (CL). Further, this inability to appropriately respond to infectious/inflammatory stimuli is driven by concomitant development of regulatory lymphoid/M/phi phenotype(s) as a response to the tissue injury (CL). Utilizing specific gene deficiency or antibodies or inhibitors we will: (1) establish which T-cell/M/phi sub-populations contribute to the suppression of cellular/Th1 lymphoid responsiveness seen in the spleen, intestine and liver following CL or CLP; (2) we will determine the role of p38 MAPK, STAT6 and SOCS-3 in the development of these regulatory cell sub-populations; (3) we will establish the role of IL-10, IL-4, TGF-beta and NO (from iNOS) in the development of these regulatory cell sub-populations in the response to CL or CLP; and (4) we will establish to what extent these changes are a result of stimulation via pattern recognition receptors (toll-family receptors, TLR) and/or apoptotic cell recognition. It is our firm belief that the results of these studies will provide information that not only will allow us to better understand the pathobiology of sepsis-induced immune dysfunction, but also its attenuation.

Grant: 2R01GM046700-10A2
Program Director: CHIN, JEAN
Principal Investigator: STRAUCH, MARK A
Title: AbrB binding to developmentally controlled promoters
Institution: UNIVERSITY OF MARYLAND BALT PROF SCHOOL BALTIMORE, MD
Project Period: 1992/02/01-2007/04/30

DESCRIPTION (provided by applicant): The *Bacillus subtilis* AbrB protein is a key global regulator that adjusts gene expression to fit metabolic needs in suboptimal environments, in the face of stress and during the initial stages of the developmental process of sporulation. In addition to preventing inappropriate expression of stationary phase associated functions during rapid growth, evidence suggests that AbrB also plays roles in modulating catabolite repression and could affect growth-rate regulation of translational components. AbrB is a transcriptional regulator whose N-terminal domain is paradigmatic for a new class of DNA-binding motif that primarily recognizes subtle three-dimensional DNA structures that are assumed by a subset of varying base sequences. Over 40 operons encoding a wide array of metabolic functions, including essential sporulation genes and genes responsible for production of antimicrobial compounds, are known to have AbrB binding sites, usually in the promoter regions. At least 25 other regulatory proteins present in *Bacillus*, *Clostridium*, *Listeria* and *Carboxydotherrmus* species (including pathogenic species) show extensive amino acid identity and homology to the DNA-binding domain of AbrB. Elucidation of the factors responsible for flexible AbrB binding specificity will provide insights in protein-DNA recognition mechanisms and how a cell can economically use a single protein to coordinate a variety of stress responses and developmental options. Examining the roles played by specific residues and regions in the protein, and comparing and contrasting properties specified by sequence variations in AbrB homologs, will provide significant insights into macromolecular interactions that are exploited by proteins having this flexible binding motif in order to achieve broad, but specific, DNA recognition properties. The relationship of AbrB structure to DNA-binding properties, and the precise role played by specific amino acid residues present in and near the binding surface, will be probed by a combination of mutant selection, mutant analysis, and examination of the binding domains from selected AbrB homologs. The hypothesis that the carboxy-terminal domains of these proteins are primarily higher order multimerization domains will be tested using a variety of approaches including genetic, biochemical and biophysical analysis of mutant proteins and construction of hybrid proteins having either intact or truncated C-domains fused to the DNA-binding domains of other AbrB homologs, or to the ? Repressor. Information gained by these investigations will be a crucial step towards an ultimate goal of utilizing the unique properties of the AbrB motif in order to design, or directly select, specific variants that specifically bind any desired DNA target.

Grant:	2R01GM048167-10A2	
Program Director:	SHAPIRO, BERT I.	
Principal Investigator:	MISRA, RAJEEV	PHD
Title:	Assembly of E Coli Outer Membrane Proteins	
Institution:	ARIZONA STATE UNIVERSITY	TEMPE, AZ
Project Period:	1992/08/01-2007/04/30	

DESCRIPTION (provided by applicant): Protein folding and assembly are central biological processes that must occur correctly for the proper functioning of all living cells. Many devastating human diseases, including neurodegenerative Alzheimer and prion diseases, are the consequence of disarrayed protein folding and assembly. A relatively large amount of cellular activity is dedicated to ensure the correct folding and assembly of proteins. In the event of misfolding, proteins are driven to aggregation and degradation pathways. Degradation of misfolded proteins is crucial because they may form toxic aggregates, which can interfere with normal cellular functions. Assembly factors that minimize aggregation (chaperones and foldases) or remove aggregates (proteases) are therefore complementary cellular activities that are regulated in response to the protein-folding status of the cell. Studying the assembly of membrane proteins has been a challenging task owing to their complex folding behavior. However, a recent explosion in the structural resolution of many membrane proteins, including those included in this study, has given a renewed impetus to the field of membrane protein biogenesis. The proposed research is directed at understanding the assembly of a unique OMP of E. coli, TolC, which folds into a novel three-dimensional structure. The TolC protein carries out several medically and physiologically important functions, including antibiotic efflux and toxin secretion. This research will identify and characterize intragenic and extragenic factors that contribute to TolC's assembly into trimeric barrels composed of alpha-helices and beta-strands. These aspects will be studied through exploiting genetic, molecular, and biochemical methods. The available data show that TolC follows an assembly pathway distinct from all other OMPs studied so far, thus providing an opportunity to uncover novel principles governing OMP targeting and assembly.

Grant: 2R01GM049711-09A1
Program Director: SHAPIRO, BERT I.
Principal Investigator: MONTAL, MAURICIO S PHD
Title: Modular Design of Voltage-Gated Channel Proteins
Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO LAJOLLA, CA
Project Period: 1993/08/10-2007/07/31

DESCRIPTION (provided by applicant): The ultimate goal is to understand the fundamental principles underlying the sequence-structure determinism, a major unsolved issue in contemporary biology. The immediate objective is to approach this question by protein design and produce a voltage-gated channel from its constituents voltage sensor (VSM) and pore (PM) modules, to retrieve its function after reconstitution in lipid bilayers, and to determine its three-dimensional structure in membranes by a combination of solution and solid-state NMR spectroscopy. The choice of voltage-gated channels is based on their importance as key control elements of cellular excitability, the mechanism underlying voltage-sensing is not fully understood, and a structure at atomic resolution is not available. A structure for the PM of *S. lividans* KcsA and of *M. thermoautotrophicum* MthK is available. The structure of the VSM remains elusive, yet this is the unique element that endows voltage-gated channels with the ability to couple a transmembrane voltage to channel opening. This is what needs to be done and what the proposed work intends to achieve. The specific aims for the revised application are focused on providing structures of the VSM and the full channel containing both VSM and PM modules of prokaryotic and designed channel proteins. Function is established by reconstitution of purified proteins in lipid bilayers and by expression of gene products in mammalian cells. Protein structure is determined by multidimensional NMR spectroscopy of isotopically labeled proteins in deuterated lipid micelles and by solid-state NMR in oriented phospholipid bilayers. The ultimate test of a successful design is recapitulation of biological function of the whole protein by assembling it from VSM and PM and determining its structure. These advances may contribute valuable insights to understand mechanisms of disease and provide structural blueprints for drug design.

Grant: 2R01GM049871-10
Program Director: JONES, WARREN
Principal Investigator: HELLINGA, HOMME W PHD
Title: Structure-based design of protein function
Institution: DUKE UNIVERSITY DURHAM, NC
Project Period: 1994/04/01-2007/05/31

DESCRIPTION (provided by applicant): Recent advances in computational, structure-based protein design methods, developed in my laboratory, successfully predict mutations that drastically alter the ligand-binding specificity of receptor proteins. Using these design techniques, several members of the E. coli periplasmic binding protein (PBP) superfamily that normally bind sugars or amino acids have been converted into receptors that recognize chemically diverse ligands with high affinity and specificity. Introduction of fluorescent and electrochemical reporter groups has permitted the engineered PBPs to be used as reagentless optical or bioelectronic biosensors. The receptors also can be re-introduced into E. coli where they control synthetic signal transduction pathways that mediate transcriptional activation response to non-natural, extracellular chemical signals. These early results are highly encouraging and suggest that the computational design of a wide variety of biological functions can be contemplated. However, in order for this capability to become a reality, it is necessary to further develop the computational design techniques, and to extend them to dealing with binding sites of increasing complexity, such as protein-protein and protein-DNA interactions. The ability to engineer proteins with a high degree of precision and sophistication has numerous biomedical applications. I propose to further develop and experimentally test the computational design techniques for manipulating molecular recognition in proteins, using specific, biomedically relevant applications as design targets that guide the choice of receptor systems and ligands that will be engineered. The tasks identified below are therefore intended to have clear practical applications, illustrating the potential wide-ranging utility of computational protein engineering to the biomedical sciences, while at the same time exploring basic scientific questions regarding molecular recognition in proteins. Aims 1-3 are focused on the development of receptors with drastically altered ligand-binding properties. These explore different applications in clinical science (Aim 1), pharmacology (Aim 2), and cell biology (Aim 3). From a basic science point of view, they will allow us to develop the techniques for engineering binding sites, and to explore scaffolds other than the PBPs (Aim 2). Aim 4 is intended to extend the design technique to much more complex systems.

Grant: 2R01GM050514-10
Program Director: TOMPKINS, LAURIE
Principal Investigator: HEYDUK, TOMASZ PHD CHEMISTRY
Title: INTER & INTRAMOLECULAR COMMUNICATIONS IN TRANSCRIPTION
Institution: ST. LOUIS UNIVERSITY ST LOUIS, MO
Project Period: 1994/08/01-2007/07/31

DESCRIPTION (provided by applicant): Transcription initiation is a major point of regulation of cellular processes. The long-term goal of this application is to obtain a detailed understanding of all steps involved in initiation of transcription, a process in which DNA-dependent enzyme RNA polymerase first locates promoter and in subsequent isomerization step melts a segment of DNA duplex in the vicinity of transcription start point to expose the template strand of DNA. Understanding transcription at a molecular level will be important for advancing the basic knowledge of this fundamental cellular process. Additionally, bacterial transcription machinery is an attractive target for drug discovery due to a remarkable conservation of structural and functional properties among bacterial RNA polymerases. Understanding bacterial transcription will thus aid in discovery of new antibiotics, an important health related issue due to the increasing problems with drug resistant microorganisms. The proposal is focused on E. coli RNA polymerase a subunit that in recent studies was shown to play intimate roles in all steps of transcription initiation. Three aims addressing fundamental issues concerning the role of this subunit in finding and melting of promoter DNA by RNA polymerase will be pursued: Aim #1. To determine molecular mechanism of initiation of transcription bubble formation. Aim #2. To determine functional roles of sigma region 4 - beta subunit "flap" domain contact. Aim #3. To determine the relative importance of sigma - DNA contacts for promoter search by RNA polymerase. Molecular events of transcription initiation are complex and involve large multi component complexes. Thus, a multi disciplinary approach combining biophysical (fluorescence and mass spectroscopy), biochemical, and molecular biology methods will be used. It is expected that a detailed understanding of the role of several protein-protein and protein-DNA contacts in RNA polymerase function will be obtained.

Grant: 2R01GM050895-09
Program Director: ANDERSON, JAMES J.
Principal Investigator: GROSSMAN, ALAN D PHD
Title: Quorum sensing and gene expression in *Bacillus subtilis*
Institution: MASSACHUSETTS INSTITUTE OF TECHNOLOGY CAMBRIDGE, MA
Project Period: 1994/05/01-2007/08/31

DESCRIPTION (provided by applicant): Cell-cell signaling controls many processes in the biological world, including development, pathogenesis, growth, mating, and transformation. Signaling processes are often mediated by factors (e.g. hormones, pheromones, neurotransmitters) that are produced by some cells and sensed by others. In bacteria, the ability to sense and respond to high population density is a type of cell-cell signaling often referred to as quorum-sensing. Cells produce extracellular signaling molecules that accumulate as population density increases, and a physiological response occurs at a critical density. The long-term goal of this project is to understand how *Bacillus subtilis* modulates gene expression and development in response to environmental conditions, with particular focus on aspects of peptide and cell-cell signaling. The major pathway for quorum sensing in *B. subtilis* involves activation of the transcription factor ComA, a response regulator that is active when phosphorylated. The activity of ComA is modulated by at least two different peptide signaling molecules that accumulate in culture supernatant as cells grow to high population density. A major challenge is to elucidate the range of cellular processes that are controlled by cell-cell signaling in a single species. This includes characterizing the genes that are regulated in response to population density, identifying the signaling molecules and pathways, and characterizing the web of overlapping interactions between responses to population density and other physiological signals. We will investigate these issues by characterizing the ComA-dependent quorum response, by characterizing other genes that are likely to be involved in cell-cell signaling, and by testing directly for and characterizing additional cell density-regulated responses. Central to this project is the use of DNA microarrays to characterize mRNA levels under a variety of conditions and in a variety of mutants. Our studies on quorum sensing and gene expression in *B. subtilis*, are relatively simple, experimentally accessible microbe should provide insights into general mechanisms of cell-cell signaling, signal transduction, and regulation.

Grant: 2R01GM051127-09
Program Director: ANDERSON, JAMES J.
Principal Investigator: FISHER, SUSAN H
Title: Regulation of Nitrogen Metabolism in *Bacillus subtilis*
Institution: BOSTON UNIVERSITY MEDICAL CAMPUS BOSTON, MA
Project Period: 1994/09/01-2007/06/30

DESCRIPTION (provided by applicant): A fundamental question in cellular physiology is how cells recognize and respond to changes in their environment. The applicant's overall goal is to understand the network of regulatory systems controlling the utilization of nitrogen compounds in the model low G+C Gram-positive bacterium, *Bacillus subtilis*. These studies will provide insight into how nitrogen metabolism is controlled in the agriculturally and commercially important *Bacillus* spp. as well as in important low G+C Gram-positive pathogens such as *Staphylococcus*, *Streptococcus* and *Enterococcus*. Two very similar global transcription factors, GlnR and TnrA, are responsible for the activation of gene expression during nitrogen-limited growth in *B. subtilis*. The activity of TnrA is regulated by a novel signal transduction system where the feedback-inhibited form of GS binds TnrA, blocking its DNA binding activity. One goal of this project is to understand the molecular mechanism responsible for the protein-protein interaction between TnrA and GS. Generalized and site-directed mutagenesis will be performed to identify amino acid residues required for feedback inhibition of GS and for the interaction between TnrA and GS. The stoichiometry and equilibrium binding constant of the interaction between GS and TnrA will be determined. The TnrA binding site will be defined by protein footprinting. All the available genetic evidence indicates that the feedback-inhibited form of GS also regulates the activity of GlnR. It will be determined whether GS directly binds to GlnR, activating its DNA binding activity. Alternatively feedback inhibited GS may indirectly regulate GlnR by a partner switching mechanism. Biochemical and genetic approaches will be used to search for factors required for the regulation of GlnR activity by glutamine synthetase.

Grant: 2R01GM051753-09
Program Director: ANDERSON, RICHARD A.
Principal Investigator: LOVETT, SUSAN T PHD
Title: Replication Associated Genetic Rearrangements
Institution: BRANDEIS UNIVERSITY WALTHAM, MA
Project Period: 1994/08/01-2007/03/31

DESCRIPTION (provided by the applicant): In recent years evidence has accumulated that replication forks frequently arrest and require repair to be restarted. Recombinational processes therefore underpin replication to permit replication of the entire chromosome. Our study of genetic rearrangements that are induced by defects in the DnaB replicative helicase of *E. coli* has revealed a requirement for the RadA/Sms protein. In several recombination and DNA repair assays, the *radA* gene plays a role redundant with *ruvABC* and *recG*, genes that encode Holliday junction processing proteins. RadA therefore most likely acts to stabilize or process recombination intermediates. RadA is a ubiquitous eubacterial protein that shares sequence similarity to the RecA/Rad51Dmc 1 strand exchange factors and to Lon protease. Our first specific aim is to characterize the biochemical properties of purified RadA protein for DNA binding, strand exchange, protease and recombination intermediate cleavage activities. The elucidation of its biochemical properties should reveal how RadA acts in recombination and DNA repair. Three areas of replication fork repair in bacteria remain incompletely understood: the replication checkpoint response, the influence of cellular localization of the chromosome and proteins on repair and the orchestration of assembly and disassembly reactions during repair. Our second aim seeks to clarify these areas by genetic analysis of replication fork repair in *E. coli*. Sensitivity to hydroxyurea, an inhibitor of Ribonucleotide reductase has been used to identify fork repair and replication checkpoint mutants in yeast. Our pilot screen for HU-sensitive mutants of *E. coli* has revealed a signal transduction protein that may regulate a cell division checkpoint and further characterization of this mutant is proposed to test this hypothesis. Several interesting mutants with *recA*-synthetic DNA damage or viability phenotypes have been isolated and will be characterized. Both traditional and genomic-based mutant analyses are proposed. Proteins known to control replication initiation and chromosome localization will also be investigated for effects on fork repair. Our third aim is to perform micro array analysis of *E. coli* genomic transcription in response to replication fork arrest or damage. These may reveal novel functions involved in the cellular response to replication fork arrest. This aim complements the second by identifying candidate genes that may control tolerance or repair of fork damage. Furthermore, mutants identified in the second aim can be screened for effects on global gene expression after conditions of replication arrest.

Grant: 2R01GM054158-05
Program Director: RHOADES, MARCUS M.
Principal Investigator: COHEN, STANLEY N
Title: Mechanisms of RNA Decay in Bacteria
Institution: STANFORD UNIVERSITY STANFORD, CA
Project Period: 1996/07/01-2007/03/31

DESCRIPTION (provided by applicant): The ability to degrade mRNA and re-use its component nucleotides is essential to viability of all living cells. There is increasing evidence that mRNA decay is a coordinated multistep process that is dynamically regulated in response to changes in cell physiology. However, limited information currently is available about the features of ribonucleases that determine their mode of action, their relative activities, and their response to physiological events. Similarly there is incomplete knowledge of the proteins and non-coding RNAs that can interact with ribonucleases and/or their substrates to modulate ribonucleolytic activity and specificity. The long-term objective of the proposed research is to achieve a better understanding of the mechanisms that regulate RNA decay in bacteria. The specific aims for the next project period are: 1) to elucidate the structural features of the RNase E and RNase G proteins that determine their specificity and mode of action, 2) to identify and investigate the effects of other cellular proteins that interact with these ribonucleases, regulate their actions, and allow them to respond to changes in cell physiology, and 3) to identify transcripts whose degradation by these ribonucleases is differentially affected by physiological changes, identify the regulatory proteins and/or small RNAs that mediate these effects, and elucidate the mechanisms of action of selected regulators. The research is multifaceted and will employ a combination of genetic and biochemical approaches--including mutational analysis of ribonuclease structure/function relationships, microarray-based investigations of changes in RNA decay in response to physiological events, protein purification and analysis, and biochemical study of the effects of regulator proteins and/or RNAs on ribonuclease action. While these investigations are intended to elucidate fundamental aspects of bacterial RNA decay, the information obtained is likely to facilitate the more efficient use of microorganisms to express biologically and medically important proteins and also to further the development of novel antimicrobial drugs that target RNA decay mechanisms in bacteria.

Grant: 2R01GM054592-06A2
Program Director: ANDERSON, RICHARD A.
Principal Investigator: SINGER, MITCHELL H
Title: Control of Initiation of Development in *M. xanthus*
Institution: UNIVERSITY OF CALIFORNIA DAVIS DAVIS, CA
Project Period: 1997/05/01-2007/06/30

DESCRIPTION (provided by applicant): The long-term goal of these studies is to understand how extracellular signals direct cellular development and differentiation by controlling gene expression. In the gram-negative soil bacterium *Myxococcus xanthus*, environmental cues such as nutrient limitation initiate a developmental phase, which culminates in the formation of a multicellular structure, the fruiting body. We have previously demonstrated how *relA* and (p)ppGpp accumulation regulate the initiation of development in *M. xanthus*. The propagation of this signal is mediated by SdeK, a cytoplasmic histidine sensor-kinase, whose expression is controlled by (p)ppGpp levels. The overall goal of this proposal is to further define three key aspects of this signaling pathway: first, to determine how the first developmental events are activated by (p)ppGpp accumulation; second, to determine the role of SdeK in propagating the developmental signal; and third, to determine the relationship between this signaling pathway and other signaling pathways that have been identified. Therefore the Specific Aims of this proposal are: (1) identify and characterize the direct regulator(s) of the (p)ppGpp-dependent genes (designated as Class 1A genes); (2) determine the interaction between (p)ppGpp and other nutrient sensing pathways; (3) identify and characterize SdeR, the target of the SdeK kinase, and other members of the SdeK/SdeR signal transduction pathway; (4) define the genetic circuitry controlling early developmental events. The studies described in this proposal will provide new insights into how cells recognize and respond to nutrient limitation. Because most microbes live in a "feast or famine" environment, deciphering cellular survival mechanisms is crucial in understanding how these organisms adapt to their environment. The use of *M. xanthus* as a model system is important because it represents a novel and tractable member of a group of bacteria that are emerging as an important source of antimicrobials, antitumor, and antiviral activities. It has been estimated that approximately 95 percent of cellulose-decomposing myxobacteria and 55 percent of the proteolytic strains, including some *M. xanthus* strains, produce biocides useful in human and veterinary medicine, and in agricultural applications. Large multienzyme complexes termed polyketide synthases and peptide synthetases, many of which are produced during starvation, synthesize many of these compounds. Finally, the work that we propose will not only shed new insight on to how *M. xanthus* integrates information from a variety of sources to control gene expression, but will provide general information that can be applied to other less tractable systems.

Grant: 2R01GM054724-06A1
Program Director: OKITA, RICHARD T
Principal Investigator: KIM, RICHARD B MD
Title: Hepatic Drug Transporters in Drug Disposition
Institution: VANDERBILT UNIVERSITY NASHVILLE, TN
Project Period: 1998/07/01-2007/06/30

DESCRIPTION (provided by applicant): Transporters are increasingly recognized as important processes in drug disposition. More recently, functional characterization of drug uptake transporters has revealed that a family of drug uptake transporters known as the Organic Anion Transporting Polypeptides (OATPs), are critical to the cellular uptake of drugs into organs such as the liver, intestine, and brain. Studies have revealed that certain human OATPs such as OATP-C and OATP-8 may be the key hepatic drug uptake transporters, while OATP-A expression at the level of the blood brain barrier may be responsible for the CNS entry of certain drugs. We are now able to show that OATP-A is expressed in the small intestines, and may be a key transporter responsible enhancing the gastrointestinal absorption of various drugs in clinical use. It is our hypothesis that intersubject variability in the expressed level and activity of OATP transporters affects drug disposition and responsiveness. However, the extent of our knowledge regarding human OATP transporters is limited. Studies carried out from this laboratory have identified a number of single nucleotide polymorphisms (SNPs) in OATP transporters importantly associated with drug disposition and response. Accordingly, in this application, studies on the role of genetic variability in certain human OATP transporters to transporter function, both in vitro and in vivo, are outlined. Specific Aim 1 is focused studies on the in vitro functional characterization of allelic variants newly identified by this laboratory in OATP-A, OATP-8 and OATP-C. In Specific Aim 2, to better understand the interplay between OATP-mediated drug uptake versus P-glycoprotein (MDR1) or MRP2 (cMOAT)-mediated drug efflux, studies are proposed on the creation of model cell lines expressing an OATP transporter along with P-glycoprotein or MRP2, in combinations reflective of organs such as the liver, intestine and brain. In Specific Aim 3, the role of commonly occurring SNPs in OATP-C, which studies from this laboratory had shown to be functionally significant in vitro, will be tested in human subjects using the well-known OATP-C-specific substrate, pravastatin, and a newly identified substrate, rifampin, as in vivo probes for this transporter. Moreover, variability in the extent of rifampin-mediated induction of the drug metabolizing enzyme, CYP3A, among subjects with variant OATP-C alleles, will also be tested.

Grant: 2R01GM055230-06A1
Program Director: WEHRLE, JANNA P.
Principal Investigator: WOODS, ROBERT J PHD
Title: Computational Analysis of Carbohydrate Antigenicity
Institution: UNIVERSITY OF GEORGIA ATHENS, GA
Project Period: 1997/03/01-2007/04/30

DESCRIPTION (provided by applicant): Group Beta Streptococcus and Neisseria meningitidis are leading causes of neonatal sepsis and meningitis. The increasing use of carbohydrate-based conjugate vaccines is founded on the observation that antibodies against the type-specific bacterial capsular polysaccharides (CPS) are often protective, and is driven by the increasing prevalence of antibiotic resistant strains. However, the relationships between the carbohydrate sequence in the CPS and antigenicity are poorly understood. Similarly, the immune response to the CPS is structure-sensitive; some are poor immunogens and others good. The goal of this proposal is to provide an understanding of the structural features of antigenic oligosaccharides that are responsible for mediating the affinity and specificity of their interactions with antibodies. Ultimately, this information would form a basis for the rational development of more effective antibacterial vaccines. We have selected three systems (Aims 1-3) for study that display complementary levels of complexity. In Aims 1 and 2, we will use computational and experimental methods to determine the conformational properties of the bacterial CPSs from iV. meningitidis and Group B Streptococcus, both free and bound to monoclonal antibody fragments. This information will provide a structural basis for interpreting the antigenicities and antibody specificities for these systems, as well as assist in the determination of the conformation of the immunodominant regions. To aid in the development and validation of the computational methods, in Aim 3, we will examine the properties of the related anionic sugars in glycosaminoglycans (GAGs), for which considerable experimental data exist. Computational methods, such as molecular dynamics (MD) simulations, are useful aids in the conformational analysis of oligosaccharides and oligosaccharide-protein complexes; yet at present they have been developed only for neutral carbohydrates. The CPSs from N. meningitidis and Group B Streptococcus, and from many other pathogenic bacteria, contain anionic carbohydrate residues. Accurate modeling of these molecules will require the extension and validation of our existing carbohydrate force field parameters (GLYCAM). To assist in this development, we will examine a number of anionic GAGs, such as hyaluronan, chondroitin and heparin sulfate as well as heparin, free and complexed to antithrombin III, whose conformational properties have been well characterized experimentally. Validation of the MD simulations will be based on comparisons with NMR and X-ray data and binding affinity measurements.

Grant: 2R01GM055544-04
Program Director: RHOADES, MARCUS M.
Principal Investigator: WEAVER, KEITH E
Title: Study of stability genes of E. faecalis plasmid pAD1
Institution: UNIVERSITY OF SOUTH DAKOTA VERMILLION, SD
Project Period: 1999/05/01-2007/06/30

Enterococcus faecalis is a Gram-positive bacterium and a member of the normal flora of the intestinal tract. It is also capable of causing opportunistic infection and has gained notoriety recently because of a propensity to rapidly accumulate antibiotic resistance genes. These genes are commonly located on mobile genetic elements that facilitate their dissemination to other bacterial species. The objective of this proposal is to determine the molecular mechanism of action of a genetic locus, par, required for the stable inheritance of the pheromone-responsive conjugative plasmid pAD1. Par operates via a post-segregation killing mechanism, programming for death any cell that loses the plasmid. Par is regulated by a unique mechanism involving inhibition of toxin translation by interaction of its message with an antisense RNA at multiple dispersed sites and the formation of a stable complex. The goal of Aim 1 of this proposal is to determine the mechanism by which the complex is activated for translation in plasmid-free segregants. This will involve transcriptional analysis of the par RNAs, identification of sequence elements and RNases involved in complex degradation, and evaluation of sequence elements required for stability of the toxin mRNA. Expression of the par toxin, Fst, disrupts the integrity of the host cell membrane. The goal of Aim 2 of this proposal is to determine the mechanism of action of Fst. Two models of Fst function, one involving processing by cellular proteases and one involving selective action at the internal surface of the cytoplasmic membrane, will be tested. In addition, the characteristics of Fst-resistant mutants will be determined in an effort to identify the specific target of Fst. Finally, saturation mutagenesis will be performed on Fst in an effort to identify which amino acids are essential for its activity.

Grant: 2R01GM055639-06
Program Director: SOMERS, SCOTT D.
Principal Investigator: COONEY, ROBERT N MD
Title: Mechanisms of Growth Hormone Resistance in Sepsis
Institution: PENNSYLVANIA STATE UNIV HERSHEY HERSHEY, PA
MED CTR
Project Period: 1997/05/01-2007/03/31

DESCRIPTION (provided by applicant): The catabolism of protein after injury or infection results in multiple complications which prolong recovery and cause death. Nutrient intake is unable to prevent protein catabolism suggesting other factors are important. Growth hormone (GH) induces circulating insulin-like growth factor-I (IGF-I) synthesis by liver, which stimulates muscle protein synthesis. During sepsis, a 2-4 fold increase in circulating GH is seen with a 50% decrease in plasma IGF-I, a 40% reduction in muscle protein synthesis, and decreased muscle mass. The onset of GH resistance and loss of muscle in sepsis is mediated by the inflammatory cytokines, TNF and IL-1. Treating septic rats with IL-1 or TNF antagonists ameliorates the effects of sepsis on plasma IGF-I levels and muscle catabolism. The liver is the major source of circulating IGF-I, and will be the focus of this project to elucidate the mechanisms responsible for GH resistance. Postreceptor defects in GH signaling represent the predominant mechanism for GH resistance in sepsis. The magnitude of the IGF-I response to GH is determined by three distinct processes: the activation/propagation of JAK/STAT and MAP kinase signaling by GH, the regulation of IGF-I gene expression, and the termination of GH signaling. To investigate the mechanisms responsible for GH resistance, we developed a hepatocyte model of cytokine-mediated GH resistance. This novel hepatocyte model uniquely positions us to delineate the mechanisms responsible for defective GH signaling and IGF-I expression. TNF inhibits both the activation and termination of GH signaling by the JAK/STAT and MAP kinase pathways. IL-1 also inhibits the induction of IGF-I by GH, but the time course differs from TNF, potentially involving different mechanisms. The mechanisms responsible for GH resistance will be determined with in vitro studies involving TNF and IL-1, and in vivo studies in our rat model of sepsis. Our hypothesis is that cytokine-mediated alterations in GH signaling mediate hepatic GH resistance and muscle catabolism during sepsis. The specific aims are: (1) to delineate the effects of sepsis, TNF and IL-1 on the activation/propagation of GH signaling; (2) to elucidate the mechanisms by which sepsis, TNF and IL-1 regulate IGF-I gene expression; and (3) to determine the mechanisms by which sepsis, TNF and IL-1 terminate GH signaling. An understanding of how cytokines and growth factors regulate protein catabolism is important for the care of septic patients.

Grant: 2R01GM056120-05A1
Program Director: ECKSTRAND, IRENE A.
Principal Investigator: OCHMAN, HOWARD
Title: Genome Erosion in Infectious Microorganisms
Institution: UNIVERSITY OF ARIZONA TUCSON, AZ
Project Period: 1997/09/01-2007/08/31

DESCRIPTION (provided by applicant): Among the most remarkable findings that have emerged from the analysis of complete genome sequences is that bacteria that become associated with eukaryotic hosts undergo a process of genome decay, which occurs both through the mutational inactivation of genes and through deletions that encompass large regions. Gene loss and genome size reduction are prevalent among pathogens and symbionts, and appear to be as crucial as gene acquisition to the evolution and diversification of bacterial lineages. The global aim of the proposed research is to investigate the process and consequences of genome degradation by addressing two general questions: (1) What forces act in the formation, transcriptional inactivation and elimination of pseudogenes in bacterial genomes, and (2) What factors are responsible for the process of genome reduction and reorganization? Until recently, inactivated genes were thought to be exceedingly rare in bacterial genomes; however, the elucidation of complete genome sequences has revealed that many pathogenic bacteria contain hundreds of pseudogenes. By focusing on closely related pathogenic and free-living species, the proposed work will investigate how pseudogenes arise and persist in bacterial genomes. Although these inert genes have been previously thought to be of no consequence to an organism, their maintenance may well be detrimental, and we will test whether selection acts to silence certain sequences. To investigate the dynamics of bacterial genome degradation, we will develop and implement new bioinformatic methods to reconstruct the process of genome contraction and reorganization, and examine how certain initial events have influenced the evolutionary trajectory of host-associated species. Cumulatively, this research has the potential of revealing new selective mechanisms acting on bacterial genomes. And because the processes examined and questions considered by this proposal are common to diverse life forms, this work will have broad impact on the interpretation of genomic information from organisms having very different ecology, lifestyles and genome organization.

Grant: 2R01GM056128-05A2
Program Director: IKEDA, RICHARD A.
Principal Investigator: ARP, DANIEL J PHD
Title: Butane metabolism in *Pseudomonas butanovora*
Institution: OREGON STATE UNIVERSITY CORVALLIS, OR
Project Period: 2003/05/01-2007/04/30

DESCRIPTION (provided by applicant): Gaseous and liquid alkanes can serve as bacterial growth substrates. Alkane monooxygenases initiate the metabolism of alkanes by catalyzing the oxidation of the alkanes to alcohols. While metabolism of methane and liquid alkanes (e.g., octane) is well studied, much less is known about the oxidation of short-chain, gaseous alkanes. The applicant's research has focused on BMOs as representatives of this understudied group of alkane utilizers and on the complete pathway of butane metabolism. Previous results with three butane-utilizing bacteria indicated a surprising level of diversity among the enzymes that harvest butane as well as those involved in the subsequent metabolism of the alcohols. The investigators now propose to focus on just one of these bacteria, *Pseudomonas butanovora*. The specific aims of the project are: 1. Characterize the diiron containing BMO, which is similar to soluble MMO, but does not oxidize methane. 2. The applicants discovered two clusters of genes, each coding for a butanol dehydrogenase and a putative butyraldehyde dehydrogenase, which use parallel pathways of electron transport. The applicant now proposes to identify the components of these parallel pathways. 3. Previous studies indicated independent regulation of the production of BMO, butanol dehydrogenases and butyraldehyde dehydrogenases by butane and intermediates in the pathway. The investigators will examine the molecular basis of the regulation of induction of the butane pathway in *P. butanovora*. The proposed experiments may provide unique insights to the basis of substrate specificity in the broad class of diiron monooxygenases that oxidize nonpolar molecules. Studies of parallel pathways will be relevant to other metabolic pathways where toxic metabolites are necessarily produced as part of the metabolism of a growth supporting substrate.

Grant: 2R01GM056665-05A1
Program Director: DEATHERAGE, JAMES F.
Principal Investigator: HARWOOD, CAROLINE S PHD
Title: Pseudomonas aeruginosa sensing and response
Institution: UNIVERSITY OF IOWA IOWA CITY, IA
Project Period: 1998/05/01-2007/08/31

DESCRIPTION (provided by applicant): This application is to explore the function in attachment and biofilm formation of cluster II chemotaxis-like genes found in the opportunistic human pathogen *Pseudomonas aeruginosa*. *P. aeruginosa* has multiple sets of chemotaxis-like genes arranged in clusters. Genes in clusters I/V are absolutely required for flagella-mediated chemotaxis. Those in cluster IV are required for pilus-mediated motility and response. Accumulating evidence suggests that some chemotaxis-like genes from bacteria may not be primarily involved in chemotaxis or motility. Such appears to be the case for *P. aeruginosa* cluster II genes. Cluster II che-like and mcp-like genes are involved in attachment of cells to surfaces and biofilm formation. They appear to play, at most, a minor role in flagella-mediated chemotaxis. Cluster II mutants are not defective in pilus-mediated twitching motility or in swarming across solid surfaces. The long-term objective of the proposed work is to determine the mechanism by which a presumed cluster II signal transduction complex directs cells to attach to surfaces and initiate biofilm formation. In specific aim 1 *P. aeruginosa* gene chips will be used to identify sets of genes that are transcriptionally activated or repressed by cluster II che-like proteins. Other experiments will explore the possibility that a cluster II signaling complex has a major role in directly modulating the activity of a cell surface molecular machine during the stationary phase of growth. The possibility that cluster II proteins modulate the activities of other proteins through direct physical contact will also be examined. In specific aim 2 genes identified as being regulated by cluster II signal transduction or genes encoding proteins whose activities are modulated by cluster II proteins, will be inactivated by mutation. The attachment and biofilm formation phenotypes of the mutants will be analyzed. Complementary physiological approaches will be used to explore how the activities of the encoded proteins may affect biofilm formation. Cluster II genes are induced in the stationary phase of growth in a cell-density dependant manner. Specific aim 3 will explore the hypothesis that cluster II genes and some mcp genes are coordinately regulated by global regulators that control the exponential-to-stationary phase growth transition in *P. aeruginosa*. The hypothesis that cluster II proteins interact to form a signal transduction complex will be tested in specific aim 4. Phospho-transfer assays will be carried out and effects of cluster II proteins on the subcellular localization of other green fluorescent tagged cluster II proteins will be determined. *Pseudomonas aeruginosa* grows as a biofilm in the lungs of cystic fibrosis patients and it grows as a biofilm in infections caused by indwelling medical devices. The proposed work will reveal proteins and genes that are required for biofilm development by prokaryotes.

Grant: 2R01GM056933-05
Program Director: SHAPIRO, BERT I.
Principal Investigator: KAISER, CHRIS A
Title: GENETICS OF PROTEIN DELIVERY TO THE PLASMA MEMBRANE
Institution: MASSACHUSETTS INSTITUTE OF TECHNOLOGY CAMBRIDGE, MA
Project Period: 1998/02/01-2007/04/30

DESCRIPTION (provided by applicant): The activities of integral membrane proteins, such as transport proteins, are often regulated by intracellular sorting. Such sorting process can produce rapid changes in the rate that a transporter is delivered to the plasma membrane in response to an intracellular signal and thus provide a way for a cell to alter its capacity to take up small molecules from the extracellular environment in response to regulatory cues. For example, the GLUT4 glucose transporter is delivered to the plasma membrane of fat and muscle cells in response to insulin, and defects in this regulated trafficking are thought to be a root cause of non insulin-dependent diabetes. Many aspects of the intracellular sorting of GLUT4 remain poorly understood at this time. Dr. Kaiser's research group proposes to continue to study regulated sorting of general amino acid permease (Gap1) in response to the nitrogen source in the growth medium. By studying amino acid permease sorting in *S. cerevisiae* it will be possible to apply the full power of a well developed genetic organism to elucidate the mechanisms responsible for regulated sorting in the late secretory pathway. Work in the previous funding period has identified a large set of genes that control the sorting of Gap1. These genes include factors directly involved in the membrane trafficking of Gap1, such as proteins required to modify Gap1 with poly-ubiquitin, a tag found to be required for the proper sorting between Golgi and endosomal compartments. In addition, genes required to generate the proper regulatory signals for Gap1 sorting were also identified. Building on these findings the proposal is to: (i) determine how Gap1 is ubiquitinated and to identify the cellular components required for recognition of the ubiquitin tag, (ii) use a combination of genetic and biochemical methods to characterize the gene products that control Gap1 sorting and to determine their site(s) of action in the secretory and endocytic paths, and (iii) determine how nitrogen-derived signals are generated and determine how the membrane trafficking machinery responsible for sorting Gap1 decodes these signals.

Grant: 2R01GM057054-05
Program Director: SOMERS, SCOTT D.
Principal Investigator: HORTON, JURETA W PHD CARDIOVASCULAR
PHYSIOLOGY
Title: MECHANISMS OF CELL INJURY IN BURN COMPLICATED BY SEPSIS
Institution: UNIVERSITY OF TEXAS SW MED DALLAS, TX
CTR/DALLAS
Project Period: 1999/08/01-2007/07/31

DESCRIPTION (provided by applicant): Despite continued improvements in early fluid resuscitation from burn trauma, early excision and grafting, and the use of both local and systemic antimicrobials to control wound infection, burn-related loss of the dermis poses a significant risk for infection in the burn patient. Inhalation injury, intubation and the need for prolonged ventilation contribute to a significant incidence of pneumonia that often progresses to multiple organ failure, a syndrome that carries a mortality rate of 50%. A persistent clinical concern is that the development of sepsis after burn trauma may exacerbate inflammatory responses and organ dysfunction associated with the initial injury. Numerous studies describe myocardial dysfunction in trauma and sepsis, but the precise cellular mechanisms remain unclear. One common feature of myocardial depression is the accumulation of cytosolic calcium ($[Ca^{2+}]_i$) by cardiomyocytes. Time course studies during the previous funding period confirmed that burn- or sepsis-related myocyte accumulation of Na^+ precedes the rise in cellular Ca^{2+} , suggesting that cardiomyocyte accumulation of Na^+ may be a prerequisite for myocyte Ca^{2+} overload. The studies proposed herein will examine the overall hypothesis that Na^+ accumulation by the cardiomyocytes is an initial event after burn trauma or sepsis and occurs via a PKC dependent pathway; Na^+ loading, in turn, promotes myocyte Ca^{2+} loading; myocyte Ca^{2+} dyshomeostasis persists due to altered expression and function of specific Ca^{2+} transport proteins, i.e., alterations in Na^+ - Ca^{2+} exchanger and SERCA may decrease Ca^{2+} efflux from the myocytes. Mitochondrial and nuclear Ca^{2+} accumulation contribute to injury of these cellular organelles, producing free radical generation, altered DNA content and integrity, myocyte apoptosis and myocardial dysfunction. We further hypothesize that recovery from burn or sepsis-related myocardial injury and dysfunction is related to DNA excisional repair as well as myocyte replication. The proposed work focuses on five specific aims to increase our understanding of the cellular events involved in burn and sepsis-related myocardial injury.

Grant: 2R01GM057059-06
Program Director: DEATHERAGE, JAMES F.
Principal Investigator: DE BOER, PIET A PHD
Title: Assembly and Function of the Septal Ring in E.coli
Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH
Project Period: 1998/02/01-2007/01/31

DESCRIPTION (provided by applicant): Bacterial cytokinesis is mediated by the septal ring, a cytoskeletal-like organelle that is associated with the cytoplasmic membrane at the site of division. The long-term goals of the proposed research are to understand at a molecular level: i) The composition and architecture of the organelle, ii) How the ring assembles from its different components, iii) How the proper site for its assembly is determined, and iv) How the ring drives cell constriction. In E.coli, the organelle consists of at least nine essential division proteins that co-assemble in a specific order to form the mature structure. The first recognized step in the formation pathway is self-assembly of the tubulin-like GTPase FtsZ on the membrane. FtsZ polymers are bound by the ZipA and FtsA proteins, resulting in a tripartite intermediate that is required for recruitment of the other components. Septal ring assembly in the cell is controlled by MinC, an inhibitor of FtsZ polymerization. The activity of MinC, in turn, is controlled by the MinD and MinE proteins. The latter two cause MinC to rapidly oscillate from pole-to-pole, forcing FtsZ assembly to the middle of the cell. Evidence indicates that MinD, in addition to driving oscillation of MinC, also specifically targets the division inhibitor to early septal ring intermediates, which are misplaced and destined for destruction. The DicB protein of bacteriophage Kim is also a potent stimulator of MinC activity, and appears to target MinC to its substrate in a similar manner. It is proposed to elucidate the mechanisms whereby MinD and DicB help to target MinC activity, and to characterize the composition of the target(s). For this and other purposes it is proposed to develop in vitro systems wherein the properties of early septal ring complexes may be studied in their 'natural' membrane-associated state. Evidence further indicates that, in addition to the known division factors, a number of components are missing from the current septal ring model. Some of these must also be early components and be responsible for the onset of septal murein synthesis. It is proposed to identify additional septal ring components by determining the cellular distribution of several good candidates.

Grant: 2R01GM057089-05
Program Director: JONES, WARREN
Principal Investigator: PALSSON, BERNHARD O PHD CHEMICAL
ENGINEERING
Title: Genome-scale in silico Model for E.coli
Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA
Project Period: 1998/08/01-2007/04/30

DESCRIPTION (provided by applicant): The recent proliferation of "omics" (genomic, transcriptomic, proteomic, and metabolic) data has necessitated a parallel expansion in the field of systems biology to meet the rising demands for analyzing this abundance of data. Constraints-based modeling, which has proven quite successful in recent years, is an approach whereby the allowable cellular phenotypes are calculated by elimination of infeasible behaviors through the successive imposition of constraints (stoichiometric, thermodynamic and flux capacity constraints). The R01 funding program begun in 1998 has enabled us to: 1) develop genome-scale constraints-based metabolic models for E. coli, S. cerevisiae, H. influenzae, and H. pylori; 2) develop methods to analyze these metabolic networks; 3) develop methods for modeling regulation, including building a core regulatory model for E. coli; and 4) develop methods for the sequenced-based analysis of metabolic demands for transcription and translation. The proposed work presented here will build on our previous accomplishments. We propose to: 1.) Expand our E. coli metabolic model to include more E. coli metabolic gene products as they become characterized, and to expand our E. coli regulatory and transcription and translation models to genome-scale. II) Integrate these three components, resulting in a comprehensive model describing metabolism, regulation, transcription, and translation and will account for roughly 2000 of the 4,401 open reading frames found in E. coli K-12 MG1655. This integrated model will then be used to develop a data-driven model-centric database, where "omics" data can place additional constraints on the allowable solution space and can be used to calculate biological parameters. The database will also provide predictions in silico of transcriptomic, proteomic, metabolomic, and phenomic data for prospective experimental design. If the proposed program is approved and implemented, we will construct and test the most comprehensive single cell model ever. This in silico model should serve as a basis for generating highly focused hypotheses and should be extendable to other sequenced strains of E. coli.

Grant: 2R01GM057226-04
Program Director: SOMERS, SCOTT D.
Principal Investigator: TRACEY, KEVIN J MD
Title: Macrophage Inactivation in Sepsis after Trauma or Shock
Institution: NORTH SHORE-LONG ISLAND JEWISH RES MANHASSET, NY
INST
Project Period: 1999/09/30-2007/04/30

DESCRIPTION (provided by applicant): Patients who develop sepsis and multiple organ failure after surgery or trauma face significant morbidity and mortality. Our approach since originally synthesizing the macrophage-deactivating, low molecular weight, multivalent guanylylhydrazone CN1-1493, has been to use this compound as a tool to probe physiological mechanisms that inhibit systemic inflammatory mediator responses. Studies of CN1-1493 mechanism of action in animal models of endotoxemia and sepsis unexpectedly revealed a neural mechanism to inhibit systemic inflammation through vagus nerve stimulation. These surprising observations revealed a previously unrecognized neuroimmune mechanism now termed the "cholinergic anti-inflammatory pathway," which rapidly deactivates macrophages. This opened a new avenue for investigating regulation of systemic inflammation that we have pursued. Preliminary Results show that electrical stimulation of the vagus nerve specifically inhibits macrophage activation, prevents TNF synthesis in liver and heart, and significantly attenuates the systemic inflammatory response to endotoxemia. Vagus nerve stimulators are widely used in the treatment of epilepsy (they are quite safe) but their impact on immune responses was previously unknown. This application now seeks funding to pursue this line of basic physiological investigation, and to define the impact of the cholinergic anti-inflammatory pathway on regulation of systemic and organ-specific macrophage activation in vivo in standard animal models of endotoxemia and sepsis. Specific Aim 1 will establish the physiological basis for the anti-inflammatory action of vagus nerve regulation of macrophage activation in specific organs by stimulating and selectively denervating the vagus nerve input to major macrophage-containing organs in the context of 1) endotoxemia and 2) sepsis. Specific Aim 2 will assess the influence of vagus nerve stimulation on early and late systemic inflammatory cytokine responses in a clinically relevant model of hemorrhagic shock followed by sepsis. The proposed approach will address whether therapeutic, cytokine-suppressing utilization of vagus nerve stimulators can reduce mortality; or indeed cause the opposite effect by increasing susceptibility to septic complications in the traumatized, shocked host. The mechanistic insights derived from understanding how vagus nerve stimulation deactivates macrophages, and whether this strategy can be used in the milieu of endotoxemia or post-traumatic sepsis, are critical for the design of future therapies targeting the pathobiology of sepsis.

Grant: 2R01GM057773-18A2
Program Director: DEATHERAGE, JAMES F.
Principal Investigator: STOCK, JEFFRY B PHD
Title: Mechanisms of Sensory Processing in Chemotaxis
Institution: PRINCETON UNIVERSITY PRINCETON, NJ
Project Period: 1984/03/01-2007/03/31

DESCRIPTION (provided by applicant): All motile prokaryotes use essentially the same mechanism to monitor the chemistry of their surroundings and navigate toward favorable environmental conditions. The *E. coli* chemotaxis system is the best-characterized example. Sensory-motor regulation is mediated by a densely packed receptor array that is imbedded in a patch of membrane at one pole of the cell. The structure is a fibrous bundle of thousands of transmembrane alpha-helical coiled coils. Chemoattractants bind at homodimeric interfaces between alpha-helices at the outside surface of the membrane. The protein kinase, CheA, binds to the opposite end that extends into the cytoplasm. CheA catalyzes adenosine 5'-triphosphate (ATP)-dependent phosphorylation of a histidine residue within an associated histidine phosphotransfer or HPT domain. The long-term objective of the proposed research is to use *E. coli* as a model to determine the molecular logic of signal transduction pathways. How does attractant binding between coiled coil subunits at the outside surface of an *E. coli* cell control kinase activity in the cytoplasm? How are different phosphorylation-induced conformational states used to transmit information? To address these fundamental questions, the architecture of the coiled coil membrane receptor assemblies will be determined using EM and X-ray crystallographic methods. Dynamical properties and distance constraints will be characterized by fluorescence resonance energy transfer (FRET) measurements. Kinetic studies of CheA phosphotransfer reactions will provide insights concerning kinase regulation and enzymology. In addition to focusing on these structural and kinetic parameters, an effort will be made to characterize the behavioral responses of *E. coli* in complex environments in order to better assess the information processing capabilities of receptor-kinase signaling complexes. These studies will provide a foundation for understanding general mechanisms that underlie Type I receptor function in both prokaryotic and eukaryotic regulatory systems.

Grant: 2R01GM058531-05
Program Director: SOMERS, SCOTT D.
Principal Investigator: TIRUPPATHI, CHINNASWAMY PHD
Title: Thrombin, Sepsis and Mechanisms of Inflammation
Institution: UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL
Project Period: 1999/02/01-2007/01/31

DESCRIPTION (provided by applicant): The overall objective of this renewal application is to test the hypothesis that the activation of Trp channel-mediated Ca^{2+} entry in endothelial cells increases lung microvascular permeability and promotes lung edema. We have shown that thrombin activation of Proteinase-Activated Receptor-1 (PAR-1) expressed on endothelial cell surface induces an increase in intracellular Ca^{2+} , which is critical in the mechanism of increased endothelial permeability. The increase in intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) is dependent on both Ca^{2+} store depletion and the Ca^{2+} store depletion-mediated Ca^{2+} influx. We showed that the prevention of Ca^{2+} influx drastically reduced the thrombin-induced increase in endothelial permeability. We also show that the Ca^{2+} influx due to store depletion occurs through activation of the transient receptor potential (Trp) gene family of channels expressed in endothelial cells. Our supporting data show that Trp1 (Trp gene homologue) is predominantly expressed in human endothelial cells and increased expression of Trp1 augments the Ca^{2+} influx in endothelial cells. Further, we show that Src kinase activation plays an important role in the regulation of Ca^{2+} influx via Trp channels. Deletion of Trp4 gene (predominant Trp isoform expressed in mouse endothelial cells) in mouse impairs thrombin-induced increase in lung microvascular permeability. The association of Trp1 with caveolin-1 (the principal protein of caveolae) in response to thrombin is required for the Ca^{2+} influx in endothelial cells. We also show that the inflammatory cytokine, tumor necrosis factor- α (TNF α) increases Trp1 expression in endothelial cells. Based on these supporting data, we propose to (i) determine the role of Src kinase signaling in the mechanism of Ca^{2+} influx in endothelial cells via Trp channels (ii) study the role of caveolin-1 in the mechanism of Ca^{2+} influx through Trp channels and its role in the mechanism of increased endothelial permeability, and (iii) investigate the effects of inflammatory mediators, TNF α and lipopolysaccharide, on the functional expression of Trp channels and its role in the mechanism of increase in endothelial permeability. The proposed studies will utilize cell and in vivo approaches involving Src null (Src $-/-$), caveolin-1 null (cav1 $-/-$), and Trp4 null (Trp4 $-/-$) mice to address the role of Trp channels in activating Ca^{2+} influx in endothelial cells and in the mechanism of increased endothelial permeability. With the achievement of these studies, we will be able to provide new molecular insight into understanding the mechanism of vascular injury and tissue inflammation associated with states of hypercoagulation such as with sepsis.

Grant: 2R01GM058933-05
Program Director: ANDERSON, JAMES J.
Principal Investigator: LIDSTROM, MARY E PHD BACTERIOLOGY
Title: Genome-Based Analysis of Methyлотrophy
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 1999/02/01-2007/01/31

DESCRIPTION (provided by applicant): The production, interconversion, and transfer of C1 units is an important basic metabolic system in all of biology. C1 units are required for a number of biosynthetic reactions and in addition, formaldehyde is produced in a number of metabolic reactions and must be detoxified. Methyлотrophs are microorganisms capable of growth on C1 compounds as sole carbon and energy sources, and methyлотrophy can be viewed as a specialized version of the C1 metabolism found in all organisms. We have used the genome sequence of a model methyлотroph, *Methylobacterium extorquens* AM1 generated in this project as a platform to develop a new conceptual framework for how assimilatory and energy metabolism achieve dynamic balance in this organism. We propose to begin to test this model using a combination of biochemical, genetic, genomic, and modeling approaches. First, we will determine the remaining unknown details of a key part of assimilatory metabolism, the glyoxylate regeneration cycle, especially with respect to the steps generating reducing equivalents. This aim addresses the question of how reducing equivalents are balanced during methyлотrophic growth. Second, we will assess small molecule regulators of the 13-hydroxybutyryl-CoA branchpoint between two of the main assimilatory pathways, the glyoxylate regeneration cycle and PHB synthesis. This aim addresses the question of what signals are involved in regulating the flow of carbon at the main assimilatory branchpoint. Finally, we will assess expression of the genes involved in the three main assimilatory pathways at both transcript and enzyme activity levels and correlate this information with alterations in cellular pools of key intermediates and cofactors. This aim addresses the question of how carbon flow is balanced between these pathways according to cell needs. The result of this study will be a systems-level understanding of central assimilatory metabolism in methyлотrophy. These approaches will provide a model for functional genomics at the physiological level, and will create a platform for future studies of the integration of methyлотrophic assimilatory and energy metabolism and the switch between methyлотrophy and heterotrophy.

Grant: 2R01GM059026-05
Program Director: ANDERSON, JAMES J.
Principal Investigator: GREENBERG, EVERETT P PHD MICROBIOLOGY, OTTAWA
Title: Quorum Sensing in *Pseudomonas aeruginosa*
Institution: UNIVERSITY OF IOWA IOWA CITY, IA
Project Period: 1999/05/01-2007/04/30

DESCRIPTION (provided by applicant): Acyl-homoserine lactone (HSL) quorum sensing occurs in a wide variety of Gram-negative bacterial species. Details of the signals, signal generators and signal receptors have been generated at a rapid pace in the past several years. Quorum sensing functions as a global regulator of gene expression and the investigators are just beginning to explore this aspect of the signaling. *Pseudomonas aeruginosa* is a particularly useful model to study global quorum control of gene expression. The genome has been sequenced, there is a commercial GeneChip, and libraries of mapped insertion mutants are being created. The signals and signal genes have been identified, and the applicants have identified over 300 genes in the quorum-controlled "regulon." *P. aeruginosa* quorum sensing is particularly relevant as it is required for virulence of this emerging human pathogen. The goals of this project are as follows: I. To fine map the induction of "early" quorum-controlled genes. This analysis will be a step in testing the hypothesis that the strength of the binding sites for the transcriptional activators defines the precise timing of induction. II. To engineer *P. aeruginosa* strains with investigator-controllable LasR and RhlR expression modules and use the constructs to test the hypothesis that at high levels of expression the timing of all genes directly regulated by LasR or RhlR should be early. At low levels of expression only those promoters with the greatest affinity for the transcription factors should respond to signal addition. III. To study the signal receptors (transcription factors) in vitro to test models for mechanisms, which control the complex quorum gene regulatory patterns. IV. To study one particular quorum-controlled gene that is specifically expressed in stationary phase. V. To examine the role of quorum sensing in biofilm development. The *P. aeruginosa* quorum sensing system provides an opportunity to understand how a simple regulatory element can affect an ordered expression of hundreds of genes in a bacterial culture. The proposed research will serve to enrich the understanding of the mechanisms and physiological significance of quorum sensing.

Grant: 2R01GM059544-23
Program Director: DEATHERAGE, JAMES F.
Principal Investigator: DAHLQUIST, FREDERICK W
Title: Mechanistic Studies of Bacterial Chemotaxis
Institution: UNIVERSITY OF OREGON EUGENE, OR
Project Period: 1981/04/01-2004/02/29

DESCRIPTION (provided by applicant): Bacterial chemotaxis is one of the best understood signaling systems in biology. It is one of a large number of "two-component" sensory systems in bacteria that use two proteins, a histidine auto kinase (CheA) and the response regulator proteins (CheY and CheB) that are the kinase substrates that are phosphorylated on aspartate residues. Phosphorylation of the response regulator domain modulates its interactions with its target domain(s) resulting in increased or decreased affinity for the other domain, depending on the system. We propose to use modern nuclear magnetic resonance techniques and other physical methods to examine three specific aims: (1) What are the structural and energetic bases for the modulation of response regulator function by phosphorylation in CheY and in NarL. NarL regulates the choice of electron acceptor protein the bacterium expresses by acting as an activator of some genes and a repressor of others. The exposure of the DNA binding domain of NarL is regulated by phosphorylation of its response regulator domain, with interactions weakening upon phosphorylation. Conversely, phosphorylated CheY has enhanced affinity for its target FlhM. Is there a common structural and mechanism for these distinct outcomes of phosphorylation? (2) What is the structural basis for the modulation of the kinase activity of CheA. CheA forms a hetero-trimeric complex with the transmembrane chemotaxis receptors and the coupling protein CheW. Ligand binding to, and reversible methylation of, the receptor have dramatic effects on CheA activity. What role does CheW play in the coupling mechanism? Where does CheW bind to CheA? What role does CheW play in transmitting the receptor information to CheA? To answer these questions we propose to solve the structures of CheW in complex with CheA using NMR and/or x-ray crystallography. (3) How do the chemotaxis receptors transmit information? The aspartate receptor of E. coli is responsible for chemotaxis to two attractants, aspartate and maltose and for repellent responses to Co (II) ion. Maltose does not bind directly to the receptor but interacts via the periplasmic maltose binding protein (MBP). How does MBP interact with the periplasmic domain of the receptor? Is the strength of that interaction dependent on maltose binding to MBP? Where do CheW and CheA interact with the cytoplasmic domain of the receptor? We propose to use NMR and other physical methods to answer these questions.

Grant: 2R01GM059902-05
Program Director: LEWIS, CATHERINE D.
Principal Investigator: ELLENBERGER, TOM E DVM
Title: Structural Studies of Site-Specific Recombination
Institution: HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA
Project Period: 1999/08/01-2007/07/31

DESCRIPTION (provided by applicant): Phage lambda integrase (Int) is the archetype of a large family of site-specific DNA recombinases that function in the segregation of plasmids, viral and cellular chromosomes, the regulation of gene expression, and programmed gene rearrangements. These enzymes catalyze DNA cleavage and religation without the addition of high energy cofactors. They are the only enzymes known to both create branched Holliday junction intermediates and to resolve them into recombinant DNA duplexes. Recent crystal structures of the related P1 Cre recombinase and the yeast Fli protein complexed to Holliday junctions have provided many insights into the recombination reaction. In contrast to these simple recombinases, lambda Int functions in higher order structures comprising multiple DNA sites and accessory factors that bend the DNA into a compact shape. These additional interactions, which are essential for Int-catalyzed recombination in vivo, allosterically regulate the efficiency and fidelity of DNA cleavage and strand transfer. We have determined a crystal structure of a covalent Int-DNA complex that, in comparison to an earlier structure of unbound Int, reveals a DNA-mediated switch in the structure of the enzyme active site. We now propose crystallographic studies of the higher order Int-DNA complexes that will address the physical basis for the allosteric regulation of recombination through interactions of Int's two autonomous DNA binding domains.

Diffraction crystals of several of these larger Int-Holliday junction complexes have been grown and isomorphous heavy atom derivatives have been identified. The proposed crystal structure determinations, together with site-directed mutational studies of the protein subunit interfaces in these complexes, will address how DNA cleavage and ligation activities are regulated by the physical organization of the Int-DNA complexes. A physical description of the enzymatic processing of Holliday junctions and other types of DNA recombination joints is a key to mechanistic understanding of a variety of biological processes that maintain chromosome structure or create genetic diversity. The lambda Int recombination system is a model for studying the chemistry of the trans-esterification reactions and the orchestration of pairwise DNA strand exchanges that create the Holliday junction intermediate and then resolve it into recombinant DNA products.

Grant: 1R01GM063616-01A2
Program Director: RHOADES, MARCUS M.
Principal Investigator: ELLIOTT, THOMAS A BA
Title: Post-transcriptional regulation of RpoS synthesis
Institution: WEST VIRGINIA UNIVERSITY MORGANTOWN, WV
Project Period: 2003/01/01-2006/12/31

DESCRIPTION (provided by applicant): RpoS is a sigma factor, discovered and best studied in the enteric bacteria, that is important in orchestrating responses to many stresses. RpoS activity is greatly increased during stationary phase after growth in rich medium, by limitation for individual nutrients (e.g. carbon or nitrogen), by high osmolarity medium, and after entry into the eukaryotic host cell, among other stimuli. Expression of more than 50 genes responds to RpoS, and the cognate gene products act to mitigate the adverse consequences of stress for the cell. RpoS matters in the real world, where "feast and famine" is the norm. Our goal is to understand the mechanisms regulating RpoS abundance, which are poorly understood. The principal control occurs by post-transcriptional regulation of RpoS synthesis, and by regulated protein turnover. We focus here on the control of RpoS synthesis. Escherichia coli is our model organism, but the results should be broadly applicable, in two senses. First, they should illuminate the important role of RpoS in pathogenic genera such as Salmonella and Yersinia, and they will also advance our understanding of post-transcriptional gene regulation. Genetic analysis has suggested that one known RNA-binding protein, Hfq, and another possible RNA binding protein, DksA, are likely to interact with rpoS mRNA to control its expression. The small molecule "alarmone" ppGpp also has a role. The target mRNA has an antisense element that pairs with the ribosome binding site to limit translation. The function of the antisense element is counteracted in a way that requires the RNA-binding proteins and under at least some conditions, a trans-acting anti-antisense RNA. Experiments described in the specific aims utilize mainly genetic but also physical approaches: to verify the secondary structure of the rpoS mRNA, to identify the important proximal factors and their sites of action, and to determine exactly what happens to this mRNA to increase its expression.

Grant: 1R01GM063907-01A1
Program Director: SOMERS, SCOTT D.
Principal Investigator: LIU, SHU F MD
Title: Nuclear Factor Kappa B in Septic Shock
Institution: NORTH SHORE-LONG ISLAND JEWISH RES MANHASSET, NY
INST
Project Period: 2003/08/01-2008/07/31

DESCRIPTION (provided by applicant): Septic shock is a leading cause of death in the intensive care unit. A major hurdle in the clinical management of septic shock is the redundancy of mediators involved in its pathophysiology, and the inability to inhibit multiple mediators simultaneously. NF-KappaB plays a crucial role in LPS- or cytokine-activated promoter activity of over 100 genes, many of which play important roles in septic pathophysiology. NF-KappaB could be an ideal target for the development of more effective therapeutic intervention for septic shock. However, two critical questions remain to be answered. First, how critical is NF-KB in mediating the in vivo expression of multiple proinflammatory genes induced by bacterial endotoxin? Second, is blocking NF-KappaB pathway beneficial or detrimental, and when and where to block this pathway, if it is proven to be beneficial? The goal of this application is to address these two questions. Our hypotheses are that NF-KappaB is a central common pathway in LPS-induced multiple proinflammatory gene expression and LPS-triggered septic pathophysiology, and that endothelial-selective blockade of NF-KappaB activation is beneficial to the prognosis of septic shock. We will generate two double transgenic mouse lines that conditionally overexpress the degradation-resistant I-KappaBalpha (I-KappaBalpha-mt), a potent inhibitor of NF-KappaB activation, selectively in endothelial cells (VecadrTA/I-KappaBalphamt) and non-selectively in all cell types (CMVrtTA/I-KappaBalphamt) using a tetracycline-regulated gene expression system. We will study gene expression profiles of wild type and of CMVrtTA/I-KappaBalphamt mice after being challenged with LPS using microarray technique to define the in vivo function of NF-KappaB in mediating multiple proinflammatory gene expression. We will define the mechanistic role of NF-KappaB in septic pathophysiology. We will compare the efficacy of endothelial versus universal overexpression of I-KappaBalphamt in inhibiting multiple proinflammatory gene expression, in ameliorating septic pathophysiology. We will monitor and compare the mortality of wild type, VecadrTA/I-KappaBalphamt and CMVrtTA/I-KBcmt mice in LPS- and CLP-induced septic shock models. We will study the effects of endothelial selective versus universal blockade of NF-KappaB activation on host defense functions against bacterial pathogens (bacterial clearance, macrophage or PMN phagocytosis). Results from our studies will provide an experimental basis and rationale for targeting NF-KappaB pathway as a novel therapeutic strategy for the treatment of septic shock.

Grant: 1R01GM064619-01A2
Program Director: SOMERS, SCOTT D.
Principal Investigator: WONG, HECTOR R MD
Title: Genomic Analysis of Pediatric SIRS
Institution: CHILDREN'S HOSPITAL MED CTR CINCINNATI, OH
(CINCINNATI)
Project Period: 2003/08/01-2006/07/31

DESCRIPTION (provided by applicant): The systemic inflammatory response syndrome (SIRS) is a clinical concept describing a generic host response to various disease processes. The incidence of SIRS is very high in critically ill children and most clinicians have experienced the disheartening scenario wherein some children with SIRS progress to septic shock, multiple organ dysfunction syndrome (MODS), and death, despite seemingly optimal treatment. One biologically plausible explanation to account for the development of septic shock/MODS/death in some children with SIRS, but not in others, may lie in the genetic background of the individual host. That is, differences in the host's genetic background can lead to a dysregulated proinflammatory and/or antiinflammatory response, in the setting of SIRS, which can subsequently lead to septic shock/MODS/death. The central hypothesis of this proposal is based on the premise that a true understanding of the individual host's response to SIRS is dependent on genome-level investigations and that microarray technology affords an effective means to test this hypothesis. Our preliminary data indicate that RNA derived from whole blood can be used for microarray analyses to effectively study the genomic response of children with SIRS and septic shock. An additional premise of this proposal is that significant developmental differences exist in the host response to SIRS to warrant this type of investigation in an exclusively pre-pubertal population. Accordingly, the central hypothesis of this proposal is that in children with SIRS, the progression to septic shock, MODS, and/or death is, in large part, dependent on the discoverable expression patterns and interactions of a defined set of multiple gene products. To test this hypothesis we will generate a national-level, ethnically diverse, genomic database of children with SIRS, septic shock, and/or MODS (Specific Aim I). This database will be used for studies designed to elucidate the host genomic response during SIRS, septic shock, and MODS using microarray technology (Specific Aim II). Data derived from Specific Aim II studies will be partially confirmed at the level of protein expression (Specific Aim III). These data will provide the fundamental foundation for a more comprehensive understanding of these important clinical problems and thereby potentially open up new areas of investigation that will allow for the development of more specific and effective therapeutic interventions.

Grant: 1R01GM064664-01A1
Program Director: DEATHERAGE, JAMES F.
Principal Investigator: BLAIR, DAVID F PHD
Title: Biochemical & Structural Studies of MotA/MotB Proteins
Institution: UNIVERSITY OF UTAH SALT LAKE CITY, UT
Project Period: 2003/04/01-2007/03/31

DESCRIPTION (provided by applicant): Many species of bacteria swim by means of flagella, which are thin helical filaments turned by rotary motors in the cell membrane. Flagellar motility is a factor in the virulence of many human pathogens, including those that cause ulcers, syphilis, burn wound infections, and some diarrhea. The flagellar motor obtains energy for rotation from the membrane gradient of protons or, in some species, sodium ions. The molecular mechanism of rotation is not understood. Rotation must be driven by forces generated between the rotor (the rotating part) and the stator (the non-rotating part). The stator is formed from the integral membrane proteins MotA and MotB, which function to conduct ions across the membrane and to couple this ion flow to rotation. Each motor contains several MotA/MotB complexes, which function independently to generate torque. Mutational and physiological approaches have been used to identify functionally important amino acid residues in MotA and MotB, and to show that protons flowing through the motor interact with a particular aspartic acid residue(Asp32 of MotB) to drive conformational changes in the MotA/MotB complexes. Here, biochemical and structural studies are proposed that will provide a detailed picture of the conformational change that serves as the "power stroke" in the motor, and a framework for understanding the mechanism of rotation.

Grant: 1R01GM065156-01A1
Program Director: WEHRLE, JANNA P.
Principal Investigator: CAVANAGH, JOHN PHD
Title: Structural Studies of Bacterial Competence Proteins
Institution: NORTH CAROLINA STATE UNIVERSITY RALEIGH, NC
RALEIGH
Project Period: 2003/02/01-2007/01/31

DESCRIPTION (provided by applicant): Bacteria have obtained a significant portion of their genetic diversity, metabolic fitness and lethality through the acquisition of nucleotide sequences from both closely and distantly related organisms. Horizontal gene transfer produces extremely dynamic genomes, in which substantial amounts of DNA are transferred between the chromosomes of different bacterial strains. Without question, such transfers have changed the ecological and pathogenic character of bacterial species. Transformation represents one of the main mechanisms by which DNA is incorporated into the recipients genome. It involves the uptake of naked DNA from the environment and has the potential to transmit DNA between distantly related organisms. For bacteria to be transformable, they must develop the physiological state referred to as competence. Genetic competence is defined as the ability of a cell to take up free DNA from the surrounding medium. If the cell is not competent, it cannot be transformed and its growth, survival and evolution are compromised. Research into the very nature of competence addresses both very fundamental and very practical questions and concerns. What mechanisms allow bacteria to incorporate new genes to develop novel, more sophisticated mechanisms for survival? By what means is genetic diversity provided, such that a microorganism can enlarge its own genomic complement? From a health related perspective, genetic transfer via competence is becoming more implicated in the development of the pathogenic character of bacteria. There is mounting evidence that virulent attributes and antibiotic resistance can be introduced and/or enhanced in this manner. The studies proposed here will focus on mechanistic characteristics of a set of interacting proteins that form the critical regulatory network initiating competence development in *Bacillus subtilis*. The goals of the research are to provide structural, dynamic and interaction information, in order to understand the regulatory effects of the proteins MecA, ComS, ClpP/C and ComK (and their complexes). This will be achieved by a combination of high-resolution NMR spectroscopy, electrospray mass spectrometry, surface plasmon resonance and other bioanalytical techniques, along with mutagenesis and protease foot-printing investigations. The results from these studies will provide a basis for the development of models to describe the mechanism of activation of competence, not only in *B. subtilis*, but also in other important gram-positive (e.g. *Streptococcus pneumoniae*) and gram-negative (e.g. *Neisseria gonorrhoeae* and *Haemophilus influenzae*) bacteria.

Grant: 1R01GM065192-01A2
Program Director: WOLFE, PAUL B.
Principal Investigator: MCHENRY, CHARLES S
Title: DNA Replication in *Bacillus subtilis*
Institution: UNIVERSITY OF COLORADO DENVER/HSC DENVER, CO
AURORA
Project Period: 2003/08/01-2007/07/31

DESCRIPTION (provided by applicant): Decades of study have provided us with detailed information about DNA replication in *E. coli*. The explosion of bacterial genomic sequences has enabled facile exploration beyond the standard *E. coli* prototype. The objective of these proposed studies is to establish a complete replication system for a second very distant bacterium, *B. subtilis*. *B. subtilis* is ideally suited for these purposes because i. it is diverse, belonging to the low GC Gram (+) classification, evolutionarily distant (>>1 billion yr.) from Proteobacteria and *E. coli*, ii. its genome has been sequenced and it has a developed genetic system, iii. it can be grown in fermentors in large quantity making it suitable for biochemical analysis, iv) genomics indicates it is representative of the most diverse group of bacteria from a DNA replication standpoint (having two apparent distinct replicases and a number of replication proteins with no counterpart in *E. coli*, and v) it provides a model system representative of an important group of important human pathogens (streptococci, staphylococci and enterococci. The studies for the proposed grant period build upon a strong base established through unfunded preliminary studies and will focus upon establishing i. the identity of the elongation apparatus components and function, ii. the proteins required for providing RNA primers and iii. the proteins required for origin-specific initiation. Emphasis will be placed on those features of the *B. subtilis* replication system that make it distinct from *E. coli*. This will provide important information regarding the variations that can be expected between bacterial replication systems in terms of basic components, mechanisms and regulation. Understanding the biochemical details of DNA replication in an organism that contains two DNA polymerase IIIs, may provide paradigms applicable to other two-polymerase replicases including eukaryotes where Pol epsilon and delta both appear to be involved in processive chromosomal replication.

Grant: 1R01GM065216-01A1
Program Director: SHAPIRO, BERT I.
Principal Investigator: SILHAVY, THOMAS J PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: Regulation of Stationary Phase in Escherichia coli
Institution: PRINCETON UNIVERSITY PRINCETON, NJ
Project Period: 2003/02/01-2007/01/31

DESCRIPTION (provided by applicant): Growth of Escherichia coli ceases when nutrients are depleted or when secreted waste products accumulate to high levels. Under these conditions the bacteria initiate a complex developmental plan to allow extended survival. In the lab this stationary phase of the bacterial life cycle can be achieved by starvation for a single essential nutrient such as a carbon source, phosphate, or nitrogen in the form of ammonia. Implementation of the stationary phase developmental plan requires the alternate sigma factor RpoS. Previously we have identified an orphan response regulator SprE (also known as RssB or MviA). In rapidly growing cells, SprE directs RpoS for destruction by the ATP-dependent protease ClpP/X, thus maintaining this sigma factor at low levels. SprE activity is inhibited when cells are starved for carbon, and RpoS levels quickly rise. We have also shown that RpoS stimulates sprE expression. Paradoxically this regulatory feedback loop results in high levels of SprE in stationary phase cells when the protein is presumably inactive. Using a combination of genetics and biochemistry we will define the signal transduction pathway that controls SprE activity, and we will probe the functional significance of this regulatory feedback loop. We will also determine the signal transduction mechanisms responsible for the development of stationary phase when cells are starved for phosphate or ammonia, and we will determine how cells integrate the conflicting signals that can arise when cells are starved for only one of these elements. Stationary phase raises several questions of fundamental importance. Cells sense and respond to impending starvation. How do they know they are about to deplete the medium of one essential nutrient when all other essential nutrients are abundant? How are conflicting signals integrated? Finally, since RpoS is important for the pathogenesis of several bacteria, an understanding of this complex signal transduction network may reveal chinks in the armor of these pathogens.

Grant:	1R01GM065364-01A1	
Program Director:	LEWIS, CATHERINE D.	
Principal Investigator:	WHITESIDES, GEORGE M	PHD CHEMISTRY:CHEMISTRY- UNSPEC
Title:	Tools To Study Cell Motility and Ion Channels	
Institution:	HARVARD UNIVERSITY	CAMBRIDGE, MA
Project Period:	2003/02/01-2007/01/31	

DESCRIPTION (provided by applicant): This research will study two areas: Mechanisms of cell motility, and molecular action of ion channels in cell membranes. It has three specific foci: i) the motility of mammalian cells; ii) the motility of bacterial cells; and iii) electrophysiology of ion channels. The work will also develop new experimental methods for examining these biological phenomena and processes. These developments will emphasize microtools, fabricated using a combination of soft lithography, self-assembled monolayers (SAMs), microfluidics, and electrochemistry. These projects are tied together by three common themes: motility, sensing, and new, microfabricated tools for biology/biochemistry. The work will have three broad outcomes. It will clarify the molecular and cell physiological mechanisms of motility in mammalian and bacterial cells. It will provide new systems for studying ion channels, it will develop new tools to support these studies, and demonstrate these tools in the context of biological problems. These studies are relevant to a number of areas of biology in which an understanding of the mechanisms of cellular motility and cellular sensing are important; from fundamental studies of development, metastasis, angiogenesis, and infectious disease to applications in cell-based sensors and tools for high-throughput screening of leads. The work spans biology, microfabrication, and biophysics, and an important product of the research will be students broadly trained in biological and physical sciences.

Grant: 1R01GM065406-01A1
Program Director: SCHWAB, JOHN M.
Principal Investigator: BELSHAW, PETER J PHD
Title: Chemical Genomics with Natural Products Analog Libraries
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 2003/01/01-2007/12/31

DESCRIPTION (provided by applicant): The overall goals of the proposed research is to identify small molecule regulators of critical cellular processes in high-throughput screens of synthetic chemical libraries and to develop new methods to rapidly identify the cellular targets of active compounds from phenotypic screens. Chemical genomic approaches to identify the cellular functions of proteins have recently be shown to have great promise as a new tool for functional genomics and for the identification of new lead compounds and cellular targets for drug development. The proposed research aims to address two current deficiencies in the Chemical Genomic approach: modulating protein-protein interactions as targets of small molecules; identifying the cellular targets of bioactive small molecules identified in phenotype-directed screens. The specific aims of the proposed research are (1) Develop synthetic chemistry to achieve a versatile high-yield, convergent solid-phase synthesis of the streptogramin B analogs. (2) Devise novel linkers for attachment of library members to solid phase synthesis resins such that linker cleavage generates an electrophilic reactive site (Michael acceptor) on each member of the library. (3) Synthesis and evaluation of a "target identification probe" (TIP) reagent to enable the rapid identification of the cellular targets of bioactive compounds from combinatorial libraries. (4) Identification of biologically active library members and determine the cellular targets of bioactive library members using target identification probe reagents. Our integrated approach to chemical genomics includes new methods for the synthesis of electrophilic libraries, novel biological assays and a novel method for target identification as a platform for biological discovery.

Grant: 1R01GM065483-01A1
Program Director: SCHWAB, JOHN M.
Principal Investigator: SORENSEN, ERIK J PHD
Title: Synthesis of Bioactive Natural Products
Institution: SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA
Project Period: 2003/05/01-2003/08/31

DESCRIPTION (provided by applicant): The long-term objectives of this research program are (1) to achieve efficient total syntheses of natural products, which could benefit human health, through the development of powerful chemical methods and strategies, and (2) to investigate the modes of action of bioactive natural products. This grant application addresses the chemical problems posed by five biologically active natural products and its Specific Aims are: (1) to develop novel synthetic routes to the natural products harziphilone and fleephilone and characterize how these compounds inhibit binding between the HIV-1 Rev protein and the Rev Responsive Element (RRE) of viral mRNA, a required event in the replication of HIV-1 ; and (2) to achieve concise syntheses of TAEMC-161 and the steroidal antibiotics viridiol and viridin. The function of the protein HIV-1 Rev is essential for the replication of HIV and, therefore, an excellent target for therapeutic intervention. Harziphilone and fleephilone are natural products that block the critical HIV-1 Rev/RRE RNA interaction. The important bioactivities and unique structural features of these compounds make them compelling objectives for organic synthesis. A tandem process comprising three structural transformations is proposed to convert an acyclic bis-ynone to harziphilone, while an aza-ring annulation featuring the intramolecular addition of a carbon nucleophile to pyridinium or iminium ions is the cornerstone of the proposed approach to the unusual structure of fleephilone. The furanosteroid viridin and its structural relatives are cell permeable, selective inhibitors of PI 3-kinase, an enzyme essential for many biological processes, and have potential as therapeutic agents for the treatment of neoplasms in humans. As such, these compounds and other natural products that act by analogous mechanisms are prime targets for organic synthesis. This grant application describes how an efficient reaction sequence featuring metal-catalyzed cyclotrimerization, carbonyl addition, and sequential electrocyclic reactions can be integrated into a general plan for syntheses of viridiol and viridin and also illustrates a structural and potential chemical relationship between these compounds and the recently described natural product TAEMC-161.

Grant: 1R01GM065534-01A1
Program Director: LEWIS, CATHERINE D.
Principal Investigator: MUSSER, SIEGFRIED M
Title: Single Molecule Analysis of Bacterial Protein Transport
Institution: TEXAS A&M UNIVERSITY HEALTH SCIENCE COLLEGE STATION, TX
CTR
Project Period: 2003/05/01-2008/04/30

DESCRIPTION (provided by applicant): Proteins must be localized to the correct subcellular compartment for proper function. Protein translocation machineries fulfill this essential function by mediating signal-dependent transport of protein molecules across membranes. The importance of these transport processes for cell growth and development is evident from their direct role in a number of disease states including bacterial pathogenicity and protein misfolding diseases such as cystic fibrosis. Altered protein transporter structure or perturbed trafficking is responsible for a number of leukemias and cancers. Protein transport machineries ubiquitous in bacteria but absent from humans are excellent targets for antibiotic development. The goal of the proposed research is to advance our understanding of the molecular basis of protein transport mechanisms in bacteria. The Sec and Tat machineries will be examined using single-turnover stopped-flow fluorescence and single-molecule fluorescence microscopy techniques in order to dissect individual kinetic steps of transport. The specific aims of the project are: (1) to develop a real-time fluorescence-based kinetic assay for the Escherichia coli Sec machinery; (2) to determine the Sec and Tat transport times via stopped-flow fluorescence; (3) to develop a lipid bilayer system suitable for simultaneous electrical and single-molecule fluorescence measurements; and (4) to determine the transport kinetics of single Sec and Tat substrates via single-molecule fluorescence microscopy. The results will be widely applicable to our understanding of membrane transport mechanisms and will significantly advance the field of single-molecule biophysics.

Grant: 1R01GM065835-01A1
Program Director: ZATZ, MARION M.
Principal Investigator: JACOBS-WAGNER, CHRISTINE PHD
Title: Temporal and spatial control of Caulobacter cell cycle
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 2003/05/01-2008/04/30

DESCRIPTION (provided by applicant): The applicant's goal is to understand how cells can generate asymmetry and coordinate differentiation with cell cycle progression using the simple model bacterium, *C. crescentus*. In *C. crescentus*, the predivisional cell is polarized with a stalk at one pole and a single flagellum at the other pole. Every cell cycle includes an asymmetric division that gives rise to two morphologically and physiologically different daughter cells. The system affords access to genetics, biochemistry, genomics and new cytology tools to look at protein dynamics in live cells. A complex phosphorelay of two-component signal transduction proteins is at the heart of differentiation and cell cycle control in this organism. This project has three objectives: The first objective is to sort out the interactions and functions of several of the two-component proteins that participate in cell cycle control and differentiation. To do this, the investigators will use gene expression profiling, phosphorylation assays as well as cell imaging technology. The second objective is based on the recent observations that several components of this regulatory network exhibit a dynamic behavior of spatial localization, alternating between dispersed distribution and discrete accumulation at the cell pole in a cell cycle-dependent manner. To understand how their cell cycle spatial localization relates to regulation and function, the applicants will determine cis-acting sequences and factors that control polar localization of these signaling proteins. The third objective is to identify new cell cycle regulators using a genetic approach. The ultimate goal is to dissect in time and space the signal transduction mechanisms of the two-component regulatory network that controls the *C. crescentus* differentiation and cell cycle. Components of this essential cell cycle regulatory network are conserved among medically important microorganisms. Insights gained into the cellular organization of prokaryotes and the mechanisms used by them to control temporal and spatial processes will not only close a gap in our understanding of fundamentals of bacterial physiology and regulation, but will also provide a basis for rational design of new antibacterial agents.

Grant: 1R01GM066098-01A1
Program Director: CARTER, ANTHONY D.
Principal Investigator: KHODURSKY, ARKADY B PHD
Title: Structure and activity of Escherichia coli chromosome
Institution: UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN
Project Period: 2003/05/01-2008/04/30

DESCRIPTION (provided by applicant): Information stored in genes is realized through the process of transcription. While transcription of individual genes is understood as a biochemical reaction in a great deal of molecular detail, the transcription of gene ensembles cannot yet be studied in a framework of the reduced biochemical reaction. It is also likely that even if we were equipped to study ensemble activities with high molecular precision, we would miss essential macroscopic properties of the processes that govern transcription of gene ensembles in the cell. The most basic and fundamental is the process of transcription as a function of position of the genes on a chromosome. The fundamental nature of it can be underscored by the notion that at the deterministic basis of this process lays the structure of a chromosome - a critical piece of knowledge about the cell. Indeed, the relationship between structure and function is one of the fundamental principles in molecular biology. The relationship between molecular structure and function has been used very successfully to propose, understand, and verify mechanisms of action of individual protein and DNA molecules as well as their motifs. However, our understanding of organization of the higher order macromolecules, such as chromosomes, has been slow and ineffective largely due to the low-resolution capacity of indirect techniques and invasive nature of the direct ones. Whole genome DNA microarrays designed using complete sequence information made possible direct read-out of genome's activity at a single gene resolution and higher. My laboratory uses this technique to: i) directly study and model the structure of the Escherichia coli K12 (E. coli) chromosome; ii) determine how the structure of the chromosome influences its activity and vice versa. We demonstrated that variations in gene activity as a function of gene position contain useful information about the process of transcription and are not entirely random: significant short- (~ 5 kb) and long-range correlations (~ 90kb) can be detected in transcriptional spatial data series by using standard analytical tools borrowed from signal processing, information theory and statistics. I propose to extend the combined use of theoretical approaches with direct experimentation to determine: 1) the effects of internal and external perturbations, which are known to affect global chromosomal state(s), on the observed spatial correlations; 2) dynamic distribution of DNA binding proteins that are known to control global DNA properties; 3) 3-D structure of the bacterial chromosome. As a result of these studies I expect to be able to model mechanistic and structural basis for the chromosome activity. This work will reveal a new level of organization of prokaryotic genetic material and its role in bacterial physiology, and also will provide a framework for studying spatial interactions in the chromosomes of higher organisms.

Grant: 1R01GM066115-01A1
Program Director: SOMERS, SCOTT D.
Principal Investigator: COOK, CHARLES H MD
Title: Bacterial Sepsis & Reactivation of Latent Cytomegalovirus
Institution: OHIO STATE UNIVERSITY COLUMBUS, OH
Project Period: 2003/05/08-2008/04/30

DESCRIPTION (provided by applicant): Human cytomegalovirus (CMV), like other beta herpes viruses, has the ability to become latent following primary infection. CMV can later reactivate from latency, and following reactivation is a well known pathogen in immunosuppressed transplant and AIDS patients. We have recently demonstrated that critically ill surgical patients can also reactivate latent CMV, and that it appears to be a pulmonary pathogen in these patients, with worsened morbidity and possibly mortality. Unfortunately, the trigger for this reactivation remains unknown. A number of stimuli have been related to reactivation, including immunosuppression, allogeneic stimulation from transplant, cytokine stimulation, and bacterial sepsis. Using an animal model, we have recently demonstrated that intra-abdominal bacterial sepsis can cause distant reactivation of latent CMV in lungs of immunocompetent mice. Further, we have shown that this reactivation occurs in multiple organs, suggesting the possible involvement of a circulating mediator. Several inflammatory mediators released during sepsis, including endotoxin and tumor necrosis factor, have been shown to be stimulatory to CMV replication, and thus might be responsible for reactivation in this model. In addition, these mediators are known to cause significant local end organ inflammation, and our preliminary data suggests that this local inflammation may contribute to reactivation of CMV from latency. Finally, our preliminary data also suggests that reactivation of CMV from latency also causes pathology in the immunocompetent host. This proposal, therefore, will focus on determining the mechanisms by which bacterial sepsis induces reactivation of latent CMV in lungs of immunocompetent hosts. Based upon our preliminary data, we will test the hypothesis that inflammatory mediators induced by sepsis, acting either systemically or locally, stimulate CMV reactivation from latency. In Specific Aim I, we will evaluate the role of systemic and local inflammation in reactivation of latent CMV. Specific Aim II will investigate the role of nuclear factor kappa B activation in sepsis-triggered reactivation. In Specific Aim III, we will further investigate the injurious effects of pulmonary CMV reactivation. The long-term goals of our laboratory are to elucidate the critical cellular and molecular factors mediating CMV reactivation from latency in critically ill surgical patients, and to utilize these data in directing therapy to ameliorate CMV disease.

Grant: 1R01GM066751-01
Program Director: SOMERS, SCOTT D.
Principal Investigator: WELLS, CAROL L PHD MICROBIOLOGY,
MEDICAL
Title: Syndecan and Bacterial Translocation in Shock and Trauma
Institution: UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN
Project Period: 2003/02/01-2007/01/31

DESCRIPTION (provided by applicant): Normal enteric bacteria, such as *Escherichia coli* and *Enterococcus faecalis*, frequently cause complicating infections in patients with shock and trauma. A common finding in these patients is increased intestinal epithelial permeability, and experiments with cultured enterocytes have shown that bacterial adherence to and internalization by enterocytes is increased following opening of enterocyte tight junctions, exposing the enterocyte lateral surface. Syndecan-1, expressed on the basolateral surface of human enterocytes, is a cell surface transmembrane proteoglycan that expresses heparan sulfate (HS) on its extracellular domain. Our working hypothesis is that HS chains of cell surface proteoglycans, and specifically syndecan-1, may act as an enterocyte receptor or co-receptor for a variety of enteric bacteria. Preliminary data indicated that, like human enterocytes, HS and syndecan-1 are prominently expressed on the basolateral surface of cultured HT-29 enterocytes but not Caco-2 enterocytes. Experiments with HT-29 enterocytes (designed to open enterocyte tight junctions and interfere with bacterial binding to the HS chains on syndecan-1) suggested that HS may be a receptor for gram-positive but not gram-negative bacteria. The HS analog heparin, and HS itself, inhibited adherence and internalization of gram-positive *Listeria monocytogenes* by HT-29 enterocytes, and experiments with related glycosaminoglycans indicated that this inhibition was specific for HS. Additional preliminary experiments with HT-29 enterocytes indicated that heparin and HS similarly inhibited internalization of gram-positive *E. faecalis* and *Staphylococcus aureus*, but not gram-negative *Salmonella typhimurium*, *Proteus mirabilis*, and *E. coli*. Heparin did not have a noticeable effect on internalization of any bacterial species using Caco-2 enterocytes, which express low levels of HS and syndecan-1. Other preliminary experiments indicated that heparin-treated *L. monocytogenes* was less invasive in orally inoculated mice than was untreated *L. monocytogenes*. In this proposal several experimental tools are used to clarify the interactions of cultured enterocytes with a variety of gram-negative bacteria, while focusing on gram-positive *L. monocytogenes*, *E. faecalis*, and *S. aureus*. These tools include monoclonal antibodies, glycosaminoglycans, and heparin disaccharides, and two cell lines transfected to over express syndecan-1, namely ARH-77 myeloma cells and Caco-2 enterocytes. Data from in vitro studies are used to design experiments in mice (outbred and syndecan-1 knockout) to clarify the role of HS and syndecan-1 in intestinal colonization and extra intestinal dissemination of enteric bacteria. Data from these experiments may indicate that enterocytes have a receptor (related to cell surface HS and perhaps syndecan-1) involved in adherence and internalization of a variety of gram-positive bacteria including *E. faecalis* and *S. aureus*.

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Grant: 1R01GM066794-01A1
Program Director: ANDERSON, JAMES J.
Principal Investigator: ROMEO, TONY
Title: Biofilm Formation and Dispersal Mechanisms
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 2003/09/19-2007/08/31

DESCRIPTION (provided by applicant): In the natural environment, bacteria grow predominantly within sessile, matrix-enclosed communities known as biofilms, rather than as unattached planktonic cells. Biofilms protect resident bacteria from attack by the immune system, compromise antimicrobial therapy, and disperse planktonic cells, which promote the spread of infection to distant body sites. Biofilms complicate about 65 percent of recalcitrant bacterial infections. Nevertheless, the regulatory mechanisms of biofilm development, especially biofilm dispersal, remain poorly defined in any species. Our studies revealed that the RNA-binding global regulatory protein CsrA is a potent repressor of biofilm formation in *Escherichia coli* K-12 and pathogenic relatives. This effect is mediated primarily through its regulation of intracellular glycogen synthesis and turnover. Further evidence supports a role for glycogen in the synthesis of a polysaccharide adhesin. Mutations that inhibit biofilm formation were isolated in a 4-gene operon, which was cloned and found to be needed for the production of a GlcN-rich polysaccharide. Remarkably, *csrA*-induction within cells of a preformed biofilm caused extensive dispersal, releasing viable planktonic cells. This finding offers a key to unlock the biochemical and genetic bases of biofilm dispersal. The Aims of this proposal are to: 1) Characterize a novel polysaccharide adhesin needed for *E. coli* biofilm formation. The molecular genetics of its synthesis, its chemistry, and its function in biofilm formation will be assessed. 2) Investigate the genetic and metabolic determinants of polysaccharide production. The precursor-product relationship of glycogen to the polysaccharide adhesin will be examined by ELISA analysis of existing mutants and by ¹³C NMR labeling studies in conjunction with appropriate structural and regulatory mutations. The genetic regulation of its biosynthesis will be examined with reporter fusions and other approaches. 3) Biofilm dispersal will be assessed systematically by examining the effects of *csrA* induction on the polysaccharide adhesin, membrane and periplasmic proteins, LPS and the transcriptome. Results will pave the way for studies defining the mechanisms of the dispersal process. The long-range goal of these studies is to develop a full understanding of the regulatory factors, metabolic pathways and structural elements that interact in biofilm formation, and thereby obtain useful information for combating biofilm infections.

Grant: 1R01GM066859-01
Program Director: WEHRLE, JANNA P.
Principal Investigator: MERZ, KENNETH M
Title: The Bioinorganic Chemistry of Nickel: The Ureases
Institution: PENNSYLVANIA STATE UNIVERSITY-UNIV UNIVERSITY PARK, PA
PARK
Project Period: 2003/06/01-2007/05/31

DESCRIPTION (provided by applicant): The long-term goal of this project is to understand, at the molecular-level, the catalytic mechanism, stability and inhibition of ureases and through this understanding enhance our comprehension of enzyme catalysis, enzyme stability in extreme environments and the development of small-molecule therapeutics. The ureases are involved in a broad range of diseased states and a thorough understanding of the structure and function of this family of enzymes will impact our ability to modify the behavior of the ureases. The tools that will be used to reach our long-term goals are those of theoretical chemistry. The primary enzymes that will be studied are the ureases from *K. aerogenes*, *B. pasteurii* and *H. pylori*. The overarching biological questions we are addressing is how do ureases catalyze the conversion of urea to ammonia and carbamate at a rate that is 10^{14} times the uncatalyzed reaction as well as how does the urease from *H. pylori* give this bacterium the ability to survive the low-pH conditions of the gut. With the aid of theoretical tools the nature and energetics of urease-substrate interactions, urease-inhibitor interactions and reactions catalyzed by the ureases will be examined. Furthermore, we will examine the uncatalyzed decomposition and hydrolysis of urea in aqueous solution in order to understand where the ureases derive their catalytic power from. Moreover via MD simulations we will gain a better understanding of how enzymes (in particular the *H. pylori* urease can survive the low pH environment of the gut. The insights obtained into these processes will have a major impact on human health through the understanding of urease catalysis and inhibition.

Grant: 1R01GM066861-01
Program Director: MARINO, PAMELA
Principal Investigator: DAVIES, CHRISTOPHER PHD
Title: Molecular Targets in Peptidoglycan Synthesis
Institution: MEDICAL UNIVERSITY OF SOUTH CHARLESTON, SC
CAROLINA
Project Period: 2003/02/01-2007/01/31

DESCRIPTION (provided by applicant): The murein sacculus is a mesh of cross-linked peptidoglycan strands that confers rigidity to the bacterial cell wall. Beta-lactam antibiotics, which target the essential transpeptidases (penicillin-binding proteins or PBPs) that cross-link the peptidoglycan strands, are important compounds in the treatment of bacterial diseases. Unfortunately, the emergence of multiple mechanisms of antibiotic resistance threatens to make these and other antibiotics obsolete in the treatment of bacterial infections. Along with other pathogenic bacteria, antibiotic resistance in *Neisseria gonorrhoeae* is a growing problem. Penicillin and tetracycline, once the antibiotics of choice for treatment of gonococcal infections, are no longer be used due to the emergence of resistant strains. Moreover, increasing numbers of strains are now resistant to the fluoroquinolones, one of the two antibiotics current recommended in the treatment of gonorrhea. Clearly there is an urgent need to develop new antimicrobials directed both against well-known molecular targets, such as PBPs, but also against novel targets. In this proposal we describe structural and biochemical studies of three enzymes involved in peptidoglycan metabolism: a D-D-carboxypeptidase from *E. coli* (PBP 5) that serves as a model system for elucidating PBP function, an essential transpeptidase (PBP 2) from *N. gonorrhoeae* that is the lethal target of current beta-lactam antibiotics, and a lytic transglycosylase, MltA, also from *N. gonorrhoeae*, that serves as the lynchpin of the cell wall synthesizing complex. Each of these proteins has been selected to address one or more of the following aims: (a) to understand the biology of peptidoglycan synthesis, (b) to explore their interactions with antibiotics, (c) to elucidate the molecular basis for antibiotic resistance and (d) to examine their potential as targets for drug development. Studies on PBP 5 will elucidate the mechanism by which this enzyme hydrolyzes substrate and will provide a better understanding of PBP-antibiotic interactions in general. The molecular basis for antibiotic resistance in PBP 2 will be investigated by structural studies of the native enzyme and of a mutant isolated from a penicillin-resistant strain. The role of MltA as part of a multienzyme complex mediating peptidoglycan synthesis as well as its suitability as a novel target for antimicrobials will be examined by solving its crystal structure. These studies will provide a framework for future studies aimed at structure-based drug design and will provide substantial insight into the mechanisms of peptidoglycan synthesis.

Grant: 1R01GM066885-01A1
Program Director: SOMERS, SCOTT D.
Principal Investigator: SHERWOOD, EDWARD R PHD
Title: Resistance of Beta 2 Microglobulin Null Mice to Sepsis
Institution: UNIVERSITY OF TEXAS MEDICAL BR GALVESTON, TX
GALVESTON
Project Period: 2003/07/01-2008/06/30

DESCRIPTION (provided by applicant): Beta2 microglobulin knockout mice are deficient in CD8+ T cells and natural killer T cells. Compared to wild type mice, beta2 microglobulin knockout mice exhibit improved survival during sepsis caused by cecal ligation and puncture (CLP). Further depletion of natural killer cells by injection of anti-asialoGM1 confers near complete resistance to CLP-induced mortality. The mechanisms underlying these observations are unknown. However, two potential mechanisms have high probability. Firstly, CD8+ T, natural killer T and natural killer cells can impact innate immune responses by amplifying production of pro-inflammatory cytokines and chemokines. These cell populations can also cause direct cellular injury during inflammation. Therefore, it is hypothesized that CD8+ T, natural killer T and natural killer cells facilitate or directly mediate sepsis-induced mortality by amplifying the pro-inflammatory response and/or causing direct cellular injury. Secondly, beta2 microglobulin comprises the beta chain of the class I major histocompatibility complex and the non-classical antigen-presenting molecule CD1. Both of these molecules are important for presentation of self and foreign antigens, respectively, but their roles in regulating inflammatory responses during sepsis are unknown. It is further hypothesized that the class I major histocompatibility complex, CD1 and/or unrecognized beta2 microglobulin-associated molecules play a role in modulating the immune response during sepsis. This research project is designed to test these hypotheses. The following specific aims are proposed: Aim 1: To determine the specific contributions of CD8+ T, natural killer T and natural killer cells, the class I major histocompatibility complex and CD1 to the pathogenesis of lethal intra-abdominal sepsis. Mortality, bacterial counts, organ injury, cardiovascular function and acid-base balance will be measured following CLP in control mice and mice that are deficient in these cell populations and antigen-presenting molecules. Specific Aim 2: To determine the functional roles of CD8+ T, natural killer T and natural killer cells, the class I major histocompatibility complex and CD1 in regulating the pro-inflammatory response during lethal intra-abdominal sepsis. Intra-abdominal and systemic cytokine and chemokine production will be measured following CLP in control mice and mice that are deficient in CD8+ T, natural killer T and natural killer cells, the class I major histocompatibility complex and CD1. Specific Aim 3: To determine the role of cytolytic mechanisms in the pathogenesis of lethal intra-abdominal sepsis. We will evaluate expression of perforin, granzymes, Fas and FasL in control mice and mice that are deficient in CD8+ T, natural killer T and natural killer cells after CLP. The functional roles of the perforin/granzyme and Fas-FasL pathways will be determined by assessing survival, cardiovascular function, organ injury and acid-base balance in perforin-deficient and FasL-deficient mice. These studies are designed to define new mechanisms that are important in the pathogenesis of septic shock with the ultimate goal of developing new treatments for this lethal disease process.

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Grant: 1R01GM066946-01
Program Director: SOMERS, SCOTT D.
Principal Investigator: O'KEEFE, GRANT E MD
Title: Gene Polymorphisms, Inflammation and Outcome from Trauma
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2003/04/01-2008/03/31

DESCRIPTION (provided by applicant): Our overall aim is to identify and characterize genetic differences that are important to the human response to infection and inflammation. First, we will use in vivo and in vitro models of inflammation to determine the functional impact of single nucleotide polymorphisms (SNPs) in inflammation-related genes. Second, we propose to identify SNPs that will help characterize an individual patient's risk for severe infection and death after injury. This information will allow clinicians and researchers to more rationally investigate and use specific therapies aimed at reducing the devastating consequences of severe injury and infection.

Grant: 1R01GM066988-01A1
Program Director: CHIN, JEAN
Principal Investigator: MISRA, RAJEEV PHD
Title: Export & Import of Lethal Agents Mediated by TolC
Institution: ARIZONA STATE UNIVERSITY TEMPE, AZ
Project Period: 2003/08/05-2007/07/31

DESCRIPTION (provided by applicant): Transport of molecules across biological membranes is a fundamental process shared by all living cells. In Gram-negative bacteria, such as Escherichia coli, transport occurs across two membranes, of which the outer membrane represents the first permeability barrier. Outer membrane protein-mediated transport is needed to bring nutrients inside and expel unwanted metabolites and inhibitors. Ironically, toxic biomolecules and infectious agents, present in the hostile environment in which bacteria live, frequently exploit these outer membrane proteins to gain entry into the cell. The focus of this research is a unique outer membrane protein, TolC, which mediates the diffusion of a diverse group of molecules including the export of alpha-hemolysin and the efflux of antibiotics. Despite the resolution of TolC's three-dimensional structure, the role of individual residues and the mechanism by which it accomplishes the transport of a diverse group of molecules remains poorly understood. Moreover, it is unknown how TolC interacts with other proteins of the inner membrane to form the complexes that facilitate the two-way transport activity. The present study is being undertaken to examine the role of TolC in export (antibiotic) and import (colicin E1), as well as to examine its interactions with other proteins of the transport complexes. Identification of functionally critical TolC residues and the various protein-protein interactions that must occur to accomplish the diverse transport activities will be achieved through employing a combination of genetic, structural, and biochemical approaches; available preliminary data concerning all aspects of the project strongly reflect their feasibility. The presence of TolC and its homologues in other Gram-negative bacteria demonstrates the broader relevance of this unique multifunctional protein. Besides the obvious role of TolC in antibiotic resistance, its ability to mediate the secretion of a variety of virulence factors in pathogenic bacteria makes it a medically important subject of investigation.

Grant: 1R01GM067101-01
Program Director: SOMERS, SCOTT D.
Principal Investigator: HAN, JIAHUI PHD
Title: Macrophage Death and Sepsis
Institution: SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA
Project Period: 2003/01/01-2006/12/31

DESCRIPTION (provided by applicant): Overproduction of proinflammatory cytokines by macrophages and other cells is critical in the development of septic shock. On the other hand, immunocompromise of macrophages and lymphocytes that developed during the onset of sepsis was also believed to contribute to the lethality of this disease. The lifespan of macrophages and lymphocytes was modulated in sepsis and is responsible, in part, for the uncontrolled inflammatory response and immunodepression. Recent studies have shown that inhibition of lymphocyte apoptosis increased the survival rate of sepsis in an animal model. However, the effect of macrophage apoptosis on the outcome of sepsis has not been addressed. We found that macrophage apoptosis was induced in the same animal model when caspase inhibitor was administered to prevent lymphocyte death. We also found that Nur77 was induced in apoptotic macrophages and Nur77 induction is required for macrophage death. As reported by others using other types of cells, Nur77 induction requires transactivation of MEF2, but a signaling triggered by bacterial components is also required for macrophage expression of Nur77. This proposal will use the knowledge we have of macrophage apoptosis to promote and inhibit macrophage death in septic mice and thereby determine whether macrophage apoptosis positively or negatively affects the outcome in septic mice. We will also further elucidate the molecular mechanisms underlying the macrophage apoptosis. The proposed study will provide information regarding whether macrophage death should be avoided or enhanced in the treatment of sepsis. Our study will also lead to a better understanding of how macrophage apoptosis is controlled, which is needed to develop therapeutically useful strategies to selectively promote or inhibit macrophage death.

Grant: 1R01GM067152-01
Program Director: TOMPKINS, LAURIE
Principal Investigator: JOHNSON, CARL H
Title: Circadian Programs in Bacteria
Institution: VANDERBILT UNIVERSITY NASHVILLE, TN
Project Period: 2003/01/01-2006/12/31

DESCRIPTION (provided by applicant): Organisms from bacteria to humans manifest circadian (daily) rhythms that are controlled by an endogenous biochemical oscillator. Many processes, including sleeping/waking, body temperature, homeostatic functions, gene expression, cell division, and enzymatic activities, are regulated by these "biological clocks" and are important to human physiology. Psychiatric and medical studies have shown that circadian rhythmicity is involved in some forms of depressive illness, "jet lag," drug tolerance/efficacy, sleep disorders, and other aspects of human physiology. Therefore, understanding the molecular basis of circadian clocks is of fundamental biological interest and may lead to insights that will be useful in the diagnosis and treatment of disorders that are relevant to sleep, mental health, and pharmacology. Although recent breakthroughs in the field of circadian rhythms have identified a number of proteins that appear to act as clock components, we have only just begun to understand how these components interact functionally with themselves to generate precise, temperature-compensated, entrainable 24 hour oscillations. Although the specific clock proteins in cyanobacteria, Neurospora, Drosophila, and mammals are different, evidence from these diverse organisms supports a common model that proposes autoregulatory feedback loops of central clock gene expression; these observations encourage a comparative approach. This project focuses on the least-complex and most technically malleable organism in which a biological clock has been demonstrated, namely the cyanobacterium, *Synechococcus elongatus* 7942. The advantages that accrue from using this organism are its small genome, diverse genetic tools, and luciferase reporters. In this organism the monitoring of circadian gene expression is the most facile of any system presently available. Therefore, this cyanobacterial system has excellent tools for detailed molecular/genetic analyses and for clock investigations. The project will use this bacterium to address two major aspects of biological rhythmicity. First, hypotheses about its fundamental mechanism will be tested by studying the structure, function, and expression patterns of the key clock protein, KaiC. A new temperature conditional mutant will be characterized. A hypothesis that changes in chromosomal structure mediate global circadian regulation of gene expression will be tested. Second, the fitness advantage conferred by circadian control of metabolism will be characterized, including studying its mechanism.

Grant: 1R01GM067153-01
Program Director: TOMPKINS, LAURIE
Principal Investigator: ARTSIMOVITCH, IRINA PHD
Title: Mechanism of transcript elongation control by RfaH
Institution: OHIO STATE UNIVERSITY COLUMBUS, OH
Project Period: 2003/02/01-2008/01/31

DESCRIPTION (provided by applicant): Expression of many genes is limited by the ability of RNA polymerase to complete polymerization of up to a million nucleotides, making elongation to emerge next to initiation as a major regulatory step in gene expression. Several accessory protein factors that allow RNA polymerase to overcome this limitation and become "elongation-proficient have been described. The focus of this proposal, the bacterial protein RfaH, is a regulator of several long virulence and fertility operons, where it preferentially increases the expression of distal genes. We have demonstrated that RfaH binds to its recruitment sequence, ops, exposed on the surface of the RNA polymerase paused at an ops site during elongation. Following its recruitment, RfaH stimulates transcription downstream of an ops site by enhancing elongation rate and suppressing pausing. However, RfaH only modestly inhibits termination. The detailed mechanism of RfaH action, described as "antitermination", remains obscure except for the fact that it is different from those of other antiterminators such as lambdaN and lambdaQ, which have profound effects on both elongation and termination. Both the recruitment mode and the effect of RfaH on elongation are unique, thus insights into the RfaH mechanism will contribute to the general understanding of the regulation of transcript elongation in bacteria and also in eukaryotes, where RfaH homologs are implicated in elongation control and localize to the actively transcribed sites. In this proposal, we will use a combination of biochemical, genetic, and biophysical approaches to address several aspects of RfaH action. The first goal of this project is to elucidate the molecular mechanism by which RfaH affects elongation thousands of nucleotides downstream of its recruitment site. The central mechanistic question to be answered is whether RfaH travels with the elongating RNA polymerase or if it causes a conformational change in the RNA polymerase that persists for thousands of nucleotide addition steps after RfaH dissociates from the complex. The second goal of this project is to determine how universal is this mechanism by finding out whether RfaH affects transcription similarly at all sites or is targeted to a particular set of regulatory signals. The third goal of this project is to map interactions between RfaH and the transcription elongation complex, thus placing RfaH mechanism in its structural context. RfaH controls the expression of the secreted molecules, components of the cell wall, antibiotics, virulence factors, and proteins required for the mobilization of transmissible plasmids. Proposed studies will therefore positively impact research in several areas of bacterial biology and evolution, such as synthesis of extracytoplasmic components, bacterial virulence, lateral gene transfer, and emergence of pathogens.

Grant: 1R01GM067202-01
Program Director: SOMERS, SCOTT D.
Principal Investigator: ZINGARELLI, BASILIA MD
Title: PPARgamma and PPARgamma agonists in septic shock
Institution: CHILDREN'S HOSPITAL MED CTR CINCINNATI, OH
(CINCINNATI)
Project Period: 2003/07/01-2007/06/30

DESCRIPTION (provided by applicant): Severe sepsis occurs in over 750,000 patients each year in the United States and is the leading cause of morbidity and mortality in critical care units. Activation of pro-inflammatory cells by bacterial products leads to a release of inflammatory mediators that induces the systemic inflammatory response of sepsis. The production of these mediators is regulated at the transcriptional level by enhancer elements nuclear factor kappaB(NF-kappaB) and activator protein-1 (AP-1) through interactions with specific kinases. In preliminary in vitro and in vivo studies we have obtained evidence that the systemic inflammatory response of sepsis may be counter-regulated by the nuclear receptor peroxisome proliferator activated receptor-gamma (PPARgamma). Pretreatment of macrophages with the specific PPARgamma ligands 15deoxy-delta12,14-PGJ2 (15d-PGJ2) or the thiazolidinedione troglitazone inhibits pro-inflammatory mediators induced by bacterial lipopolysaccharide (LPS) and heat killed *Staphylococcus aureus* (HK *S. aureus*). Consistent with this finding, we have found that PPARgamma ligands improve survival, reduce hemodynamic alterations, cytokine production and neutrophil infiltration in lung, colon and liver in rats subjected to septic shock induced by cecal ligation and puncture. Our central hypothesis is that the nuclear receptor PPARgamma is a critical anti-inflammatory pathway and that PPARgamma activation is beneficial in septic shock. Three interrelated specific aims will test this hypothesis. (1) We will evaluate the therapeutic efficacy of PPARgamma ligands on cardiovascular derangement and organ failure during polymicrobial sepsis in vivo. The effects of 15d-PGJ2, thiazolidinediones and nonthiazolidinedione PPARgamma ligands on septic sequelae will be examined in vivo in rats subjected to cecal ligation and puncture. (2) We will identify the molecular mechanisms of the actions of PPARgamma ligands in polymicrobial sepsis in vivo. The effect of PPARgamma ligands on nuclear activation of NF-kappaB and AP-1 and their regulatory kinases will be examined. (3) We will determine the role of cyclopentenone prostaglandins and PPARgamma in regulating cell signaling and inflammatory mediator production in macrophages/monocytes challenged in vitro with LPS and HK *S. aureus*. This approach will employ pharmacologic antagonists of PPARgamma and genetic manipulations with PPARgamma dominant/negative constructs and PPARgamma deficient macrophages from Cre-lox mice. The combination of in vivo and in vitro approaches will provide a strong test of the hypothesis that activation of PPARgamma is beneficial in sepsis.

Grant: 1R01GM067542-01A1
Program Director: JONES, WARREN
Principal Investigator: ANDERSON, AMY C PHD
Title: Design of *C. parvum* and *T. gondii* DHFR-TS Inhibitors
Institution: DARTMOUTH COLLEGE HANOVER, NH
Project Period: 2003/05/01-2008/03/31

DESCRIPTION (provided by applicant): Opportunistic infections caused by *Cryptosporidium parvum* and *Toxoplasma gondii* represent life threatening diseases for immuno-compromised patients, children and the elderly. There are currently no effective treatments available for cryptosporidiosis and treatments for toxoplasmosis require the coadministration of sulfadoxine, a compound to which many patients have severe adverse reactions. The development of novel therapeutics that are highly potent and highly selective for the pathogen is of immediate importance. Crystal structures of the validated drug target, dihydrofolate reductase-thymidylate synthase (DHFR-TS), a bifunctional enzyme in protozoa, from *C. parvum* and *T. gondii*, provide essential evidence for structure-based drug design against these targets. In the first Aim of this proposal, we will design trimethoprim analogs to interact favorably with species-selective elements of the *C. parvum* DHFRTS structure. Species-selective elements will be determined by comparing crystal structures of pathogenic DHFR-TS and human DHFR. New inhibitors will be modeled into the structure of the enzyme, while accounting for ligand-induced conformational changes, and binding modes predicted. Crystal structures of trimethoprim analogs will guide future design in an iterative cycle. In the second Aim, we will improve the potency and selectivity of a promising *T. gondii* DHFR-TS inhibitor, using information from crystal structures of *T. gondii* DHFR-TS bound to lipophilic inhibitors. Designs for the improvement of *T. gondii* DHFR-TS inhibitors will take advantage of species-selective elements. In the third Aim, we will elucidate the structural basis of pyrimethamine resistance in DHFR and design potency and selectivity into a novel inhibitor of pyrimethamine-resistant *T. gondii* DHFR-TS. DHFR resistance to pyrimethamine and other antimicrobials is a threatening problem and novel therapeutics capable of inhibiting the resistant enzymes are desperately needed. We will solve a crystal structure of pyrimethamine-resistant DHFR-TS bound to a novel and exciting inhibitor that shows an inhibition constant of 350 nM against the resistant enzyme in preliminary studies. Using the structural information we will elucidate the structural basis of resistance and modify the novel inhibitor for greater potency and selectivity.

Grant: 1R01GM067624-01
Program Director: LEWIS, CATHERINE D.
Principal Investigator: RAMAKRISHNAN, VENKATRAMAN PHD
Title: Structural studies on ribosomes and antibiotics
Institution: MEDICAL RESEARCH COUNCIL CAMBRIDGE,
Project Period: 2003/08/01-2007/07/31

DESCRIPTION (provided by applicant): The ribosome is the large nucleoprotein complex that uses mRNA as the template and aminoacylated tRNAs as substrates to catalyze protein synthesis in all cells. Ribosomes consist of two subunits in all organisms, designated 30S and 50S in bacteria, which together form the 70S ribosome. The 30S subunit improves the fidelity of translation by monitoring codon-anticodon interactions, while the 50S catalyzes peptide bond formation. Both subunits act in concert during translocation, which involves the movement of mRNA and tRNA through the ribosome. Many clinically important antibiotics target the bacterial ribosome. Recent advances, including those from our own laboratory, have resulted in high resolution structures of each subunit and a medium resolution structure of the whole 70S ribosome. These structures have revolutionized our understanding of ribosome structure and function. This is a proposal to build on these advances. We have elucidated the interactions of several antibiotics with the 30S subunit, and propose to determine the structure of several important remaining ones. We shall also determine the structure of the 30S subunit in complex with initiation factors, protein S1, and with a variety of tRNA and mRNA combinations that involve non-standard pairing or modified bases at the third position of the codon, the wobble position. A special RNA called tmRNA, because it has both tRNA and mRNA-like properties, is used by the cell to rescue ribosomes stalled on defective messages. We shall determine the structure of the ribosome in complex with tmRNA in various states. We shall also crystallize complexes of the ribosome specifically arrested at various points along the translation pathway. Solution of crystal structures of these complexes will shed light on the mechanisms involved during translation, including the interaction of factors with the ribosome and conformational changes during translation. These studies will not only shed light on fundamental aspects of translation, a central process in all cells, but also reveal how many antibiotics interact with the ribosome. Such knowledge could help improve existing antibiotics and could also lead to the design of new ones.

Grant: 1R01GM067636-01
Program Director: SCHWAB, JOHN M.
Principal Investigator: TRAUNER, DIRK PHD
Title: Total Synthesis of Biologically Active Gamma-Pyrones
Institution: UNIVERSITY OF CALIFORNIA BERKELEY BERKELEY, CA
Project Period: 2003/05/02-2008/04/30

DESCRIPTION (provided by applicant): Total syntheses of the immunosuppressants SNF4435 C and D as well as the antibiotics spectinabilin and N-acetyl aureothamine are proposed. These highly unsaturated polypropionates were isolated from various strains of *Streptomyces* sp. Furthermore, synthetic approaches towards the molluscan polypropionates photodeoxytridachione and crispatene as well as the fungal immunosuppressants candelalide A and B are presented. All natural products feature a terminal gamma-pyrone moiety as a common structural motif. Polyenes consisting of an array of conjugated trisubstituted double bonds have been used as synthetic precursors of the complex polypropionates. The stereoselective assembly of these structures will be further investigated. Preliminary studies have shown that the core bicyclo[4.2.0]octadiene core of SNF4435 compounds can be formed using a highly stereoselective tandem Stille cross-coupling / electrocyclization cascade. A novel Lewis-acid catalyzed cyclization leading to the bicyclo[3.1.0]hexane core of photodeoxytridachione and crispatene has also been developed. The proposed total syntheses provide an opportunity to investigate asymmetric electrocyclization reactions. As opposed to cycloadditions and sigmatropic rearrangements, this class of pericyclic reactions has not yet succumbed to asymmetric catalysis. In a further contribution to synthetic methodology, new approaches towards gamma-pyrones are proposed. The significance of the gamma-pyrone moiety for the biological activity of the natural products will be explored. The biological mode of action of SNF4435C and D remains presently unknown. The natural products do not suppress IL-2 production, distinguishing them from FK506 or cyclosporin A. Our synthetic material and derivatives thereof will be used to isolate binding proteins and gain further insight into of the mechanism of these promising new lead compounds for drug development.

Grant: 1R01GM067644-01
Program Director: CHIN, JEAN
Principal Investigator: CHANG, GEOFFREY A PHD
Title: X-ray Structures of Small MDR Efflux Pumps
Institution: SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA
Project Period: 2003/01/01-2006/12/31

DESCRIPTION (provided by applicant): Bacterial multidrug resistance (mdr) is a significant problem in the treatment of most infectious diseases. This multidrug resistance is caused by the over-expression of drug efflux pumps that are located in the lipid bilayer of bacteria. An important class of mdr pumps in bacteria are the Small Multidrug Resistance (SMR) family of transporters. These transporters translocate hydrophobic cations through a coupling mechanism through the cell membrane using energy derived from H⁺ gradients. Recently, our laboratory has over-expressed, purified, and crystallized a full-length member of the SMR family. Our objective is to discover the molecular structural components that are involved in the translocation of multiple drug molecules through the cell membrane by SMR transporters and to understand the general transport mechanisms that confer the multidrug phenotype. A high-resolution atomic structure of a bacterial multidrug resistance SMR transporter will serve as an excellent model for other 12-TM antiporters that are involved in sugar, ion, amino acid, inorganic, and organic permeation through the membrane. An x-ray crystal structure of an SMR transporter could also provide structural information that will be useful for understanding more complicated yet homologous mammalian transporters. Our objectives are: 1. Over-expression and purification of SMR transporters and their homologs. 2. Crystallization and x-ray data collection of SMR transporters. 3. X-ray structure determination and refinement of SMR transporters. 4. Structural studies of SMR transporters concerning substrate translocation. 5. Structural studies of SMR transporters concerning substrate recognition.

Grant: 1R01GM067749-01
Program Director: JONES, WARREN
Principal Investigator: COPLEY, SHELLEY D. AB
Title: Recruitment of Enzymes to Serve New Functions
Institution: UNIVERSITY OF COLORADO AT BOULDER BOULDER, CO
Project Period: 2003/05/01-2007/04/30

DESCRIPTION (provided by applicant): Recruitment of proteins to serve new functions has played a key role in the evolution of metabolic diversity during the nearly 4 billion-year history of life on Earth. This process continues to be important as bacteria respond to novel selection pressures imposed by human activities. Of particular importance to human health is the emergence of antibiotic resistance, which can arise by recruitment of a pre-existing protein to modify a newly introduced antibiotic. In addition, the recruitment of proteins to serve new functions in the biodegradation of toxic anthropogenic compounds is critical for removal of these toxins from the environment. Although it is clear that recruitment of proteins to serve new functions has occurred, the process has rarely been studied. This proposal describes studies of the process of recruitment of enzymes to serve a variety of functions that are critical to the survival of *E. coli*. The goal of this work is identification of genes in the *E. coli* genome for which over expression or mutation restores viability in mutant strains lacking critical metabolic enzymes by encoding proteins that can be recruited to serve the function of the missing enzyme. The following classes of enzymes will be targeted: 1) enzymes that catalyze very simple reactions, such as dehydration, oxidation of an alcohol, and phosphoryl transfer; 2) enzymes that have a common structural fold and for which many enzymes with a similar fold might be recruited to replace the missing enzyme; and 3) enzymes that have an uncommon structural fold, for which more creativity on the part of the bacterium may be required to find a replacement. Further studies will determine the level of the targeted enzyme activity in the recruited protein, as well as its original function and structural fold. For selected cases, in vitro evolution will be used to attempt to improve the level of the targeted enzyme activity in the recruited protein. The outcome of this work will be an expanded view of the process of recruitment and of the catalytic plasticity available in existing protein scaffolds. This work will provide insights into how Nature has exploited this plasticity to evolve new metabolic capabilities and will provide new information about the catalytic capabilities of certain protein scaffolds that can be exploited by protein engineers to create novel catalysts.

Grant: 1R01GM067759-01
Program Director: SOMERS, SCOTT D.
Principal Investigator: BEUTLER, BRUCE A MD CLINICAL MEDICAL
SCIENCES, OTHER
Title: Mutagenic analysis of LPS responses
Institution: SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA
Project Period: 2003/09/08-2007/08/31

DESCRIPTION (provided by applicant): The LPS signaling pathway has been analyzed using a combination of biochemical and genetic methods. Yet, to the present time, not all of the proteins that participate in LPS detection have been identified. In order to find more of them, a forward genetic strategy has been used. Germline mutations are induced in mice using N-ethyl-N-nitrosourea (ENU), and the mutants are systematically screened for their ability to respond to primary challenge with LPS, as well as their ability to develop LPS tolerance. Among the first 1365 F1 and 1686 F3 mice analyzed in this manner, three transmissible mutations have been detected. An autosomal recessive mutation, Lps2, abolishes the primary LPS response but does not reside in any of the genes that are presently known to be essential for LPS sensing. Two other mutations, one dominant and one recessive, block the development of LPS tolerance. Lps2 has been excluded from more than 99% of the genome by genetic mapping. In the present proposal, we outline plans for high-resolution mapping and cloning these mutations. Moreover, since germline mutagenesis is clearly an effective means of finding the essential cellular components of LPS sensing and feedback inhibition pathways, we will extend our effort to approach saturation, cloning all mutations that show a strong phenotypic effect. The existing mutations, and all future mutations that are acquired through this forward genetic approach, will be subjected to advanced phenotypic analysis to determine the level at which they affect LPS signaling. In the case of mutations that abolish LPS tolerance, we will attempt to determine the net impact on host resistance to infection.

Grant: 1R01GM067932-01
Program Director: JONES, WARREN
Principal Investigator: MURHAMMER, DAVID W PHD
Title: Metabolic Engineering: Extending the Lifespan of Baculo*
Institution: UNIVERSITY OF IOWA IOWA CITY, IA
Project Period: 2003/05/01-2006/04/30

DESCRIPTION (provided by applicant): The goal of this proposed research is to understand the cause of baculovirus infected insect cell death. It is expected that this will lead to the development of methodologies to extend the life span of baculovirus infected insect cells and to an improved understanding of viral cytotoxicity, in general. The hypothesis upon which this research is based is that oxidative stress and cathepsin expression contribute appreciably to baculovirus cytotoxicity. Reactive oxygen species (ROS, e.g., superoxide radical (SR) and hydrogen peroxide (HP)) overexpression, while benefiting early stages of viral replication, can also lead to premature cell death through lipid oxidation that results in membrane degradation. For example, it has been demonstrated that the concentrations of superoxide radical, oxidized proteins, and oxidized lipids increase as the baculovirus infection proceeds. In addition, it has been shown that manganese superoxide dismutase (MnSOD, which converts SR to HP) overexpression extends the life span of baculovirus infected insect cells. Furthermore, baculoviruses express cathepsin that degrades cellular actin, thereby compromising cellular integrity. The specific aims that will be carried out to test the hypothesis of this proposed project are (1) evaluate the role of oxidative stress in baculovirus cytotoxicity and (2) evaluate the role of cathepsin expression in baculovirus cytotoxicity. The role of oxidative stress will be evaluated by infecting metabolically engineered cells that target superoxide dismutases (which convert SR to HP) and catalase and/or ascorbate peroxidase (which convert HP to water) expression to the intracellular locations of SR and HP accumulation, respectively. The role of cathepsin will be evaluated by infecting cells with a modified baculovirus lacking the cathepsin gene. In addition, the synergistic effects of oxidative stress and cathepsin expression will be investigated by infecting the metabolically engineered cells with the baculovirus lacking the cathepsin gene. The proposed research will advance the understanding of the role of oxidative stress and cathepsin expression in baculovirus cytotoxicity. The role of oxidative stress is particularly important since its role in viral cytotoxicity, in general, is not very well understood. It is possible that understanding this phenomenon could lead to the development of novel antiviral drugs.

Grant: 1R01GM067937-01
Program Director: JONES, WARREN
Principal Investigator: KHOSLA, CHAITAN S
Title: Metabolic Engineering of Polyketide Production in E.coli
Institution: STANFORD UNIVERSITY STANFORD, CA
Project Period: 2003/01/16-2005/12/31

DESCRIPTION (provided by applicant): Polyketide synthases (PKSs) are a family of multi-enzyme assemblies that catalyze the synthesis of numerous structurally complex and biologically important natural products. Modular PKSs, such as the 6-deoxyerythronolide B synthase (DEBS), are a particularly interesting sub-class of PKSs that synthesize complex polyketides such as macrolides. Over the past decade, there has been considerable interest in studying these megasynthases, and in exploiting their modularity and broad substrate specificity for the engineered biosynthesis of "unnatural" natural products. Most products of modular PKSs are produced by relatively uncharacterized bacteria. As a result, every time a new natural product with promising biological properties is discovered, a considerable amount of time and expense must be incurred to obtain reliable quantities of the compound from natural sources, and an even greater investment is demanded before the biosynthetic pathway becomes amenable to rational engineering. An alternative is to develop robust and generally applicable technologies for the heterologous expression of polyketides in well-characterized microbial hosts. During the past proposal period, the metabolism of the model bacterium *Escherichia coli* was engineered to produce 6-deoxyerythronolide B (6dEB), the macrocyclic core of the antibiotic erythromycin. This engineered strain of *E. coli* harbors modifications in five endogenous genes; it also contains seven new genes from three different heterologous sources. The resulting cellular catalyst converts exogenous propionate into 6dEB in quantities approaching 200 mg/L over a 5-day process. During the next 3-year proposal period, we will focus on improving and extending the properties of *E. coli* as a host of choice for the biosynthesis of natural and unnatural polyketides. This will be accomplished through a combination of molecular biological tools, metabolic engineering strategies and fermentation technology development. The Specific Aims are: I] Engineering new pathways for precursor and product biosynthesis in *E. coli*; II] Improved fermentation protocols for enhancing polyketide productivity in *E. coli*; III] Further improvements in polyketide productivity of *E. coli* using functional genomic and metabolic engineering approaches; & IV] Heterologous production of two new complex natural products in *E. coli*. The implications of this research are 3-fold. First, given the availability of scalable protocols for fermenting *E. coli* to overproduce bioproducts, the ability to synthesize complex polyketides in this heterologous host will bode well for the practical production of these expensive bioactive natural products as well as their engineered derivatives. Second, the use of *E. coli* as a host for polyketide production opens the door for harnessing *E. coli* to engineer modular PKSs using directed and random approaches. Finally, the project is a good opportunity to train students at the interface of metabolic engineering & natural product biosynthesis.

Grant:	1R01GM067955-01	
Program Director:	ANDERSON, JAMES J.	
Principal Investigator:	WASSARMAN, KAREN M	PHD
Title:	Regulation Of Transcription by 6S RNA	
Institution:	UNIVERSITY OF WISCONSIN MADISON	MADISON, WI
Project Period:	2003/05/01-2008/04/30	

DESCRIPTION (provided by applicant): Regulated transcription is one of the principle mechanisms to alter gene expression in response to environmental stimuli. Intensive study over more than 3 decades has provided a detailed understanding of the transcriptional machinery as well as many of the signals to which it responds. A novel mechanism for altering transcription, which uses the RNA-regulator 6S RNA, recently has been discovered. Therefore, a detailed understanding of the role of 6S RNA in transcriptional regulation is instrumental for full comprehension of how appropriate cellular gene expression is maintained. Cells with altered 6S RNA levels are decreased in their ability to survive in stationary phase, indicating 6S RNA has a major impact on cell physiology. Many small RNAs in bacteria are utilized to optimize cellular responses to unfavorable nutritional or chemical environments through post-transcriptional regulation of gene expression. 6S RNA expands this list to include regulation in response to late stationary phase, as well as to include a unique method of regulation at the level of transcription. This proposal focuses on answering three questions regarding 6S RNA function and activity: 1. How global are 6S RNA effects on gene expression which will be addressed by identification of the 6S RNA regulon; 2. What are the physiological consequences of 6S RNA mediated changes in gene expression which will be addressed through genetic analysis of phenotypes associated with loss of 6S RNA; 3. How 6S RNA changes the transcriptional machinery and its activity, which will be addressed through biochemical approaches.

Grant: 1R01GM068061-01
Program Director: JONES, WARREN
Principal Investigator: KECK, JAMES L PHD
Title: Structure and Function of Bacterial RecQ Protein
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 2003/05/01-2008/04/30

DESCRIPTION (provided by applicant): The goal of this proposal is to determine the molecular mechanisms used by RecQ DNA helicases. RecQ involvement in DNA replication, recombination, and repair has been demonstrated in both bacteria and eukaryotes, but the precise roles played by this family of helicases remain unclear. Their importance, however, is unambiguous -- mutation of one of three human RecQ genes leads to genome instability and an elevated predisposition to cancer in Werner's, Bloom's, and Rothmund-Thompson syndromes as well as to premature aging in Werner's and Rothmund-Thompson syndromes. RecQ proteins, therefore, represent a paradigm linking genome stability to disease states as well as for understanding the connections between DNA replication, recombination, and repair. This makes a precise, molecular understanding of the mechanisms of the RecQ family important to both basic and medical science. The experiments described in this proposal aim to elucidate the biological functions of RecQ proteins by resolving their molecular mechanisms of action in vitro and in vivo. The approach focuses biochemical, high-resolution structural, and genetic methods on bacterial RecQ proteins to delineate the structure-function relationship that supports activity in the enzyme. These experiments include: (1) a determination of the roles of the conserved domains within the RecQ family (helicase, RecQ-Ct, and HRDC domains), (2) elucidation of the quaternary and tertiary structures of RecQ, and (3) identification of RecQ-containing protein complexes in bacterial cells and a study of the effects of interacting proteins on RecQ activity in vitro. A multidisciplinary approach will allow biochemical links to be made between the cellular roles of RecQ proteins and their molecular mechanisms of action.

Grant: 1R01GM068451-01
Program Director: PREUSCH, PETER C.
Principal Investigator: WEINER, JOEL HIRSCH BS BIOCHEMISTRY
Title: Structures of Bacterial Membrane-Bound Oxidoreductases
Institution: UNIVERSITY OF ALBERTA EDMONTON, AB
Project Period: 2003/09/01-2007/08/31

DESCRIPTION (provided by applicant): Only 0.36% of the 18,412 proteins in the Protein Data Bank are integral membrane proteins, yet membrane proteins comprise approximately 25% of the genome and are linked to a multitude of human diseases. Although the isolation, crystallization and NMR solution structure determination of membrane-bound proteins is viewed as difficult, new techniques are emerging to solve the technical problems. We will combine our expertise in cloning, expression, purification, X-ray crystallography and NMR to focus on structure determinations of two very well characterized E. coli membrane-bound oxidoreductases: nitrate reductase A (NarGHI) and DMSO reductase (DmsABC). These integral membrane-bound bacterial enzymes are complex multi-subunit proteins which participate in energy conservation. In addition, we will extend our research to two novel membrane-bound oxidoreductases: YedYZ and YdhXU, identified by genome analysis, which we believe also participate in energy conservation and have properties commensurate with our aims. All contain an array of cofactors including b-type hemes, iron sulfur clusters and molybdopterin cofactors which are essential for energy conservation in all living organisms. We have shown that NarGHI is ideal for the investigation of membrane protein overproduction, purification and crystallization and we believe that DmsABC is also an excellent candidate for investigation based on our intensive studies of this enzyme. The individual subunits NarI, YedZ and YdhU are excellent candidates to develop emerging multidimensional NMR techniques. Together our team will develop techniques that will be applicable to a range of membrane-bound proteins. Our specific aims are to: (i) complete the atomic resolution structure of NarGHI utilizing our existing crystals that diffract to 1.9Angstrom resolution and selenomethionine labeled protein crystals, (ii) to develop improved over-expression and purification protocols for DmsABC to produce quantities of protein which will be subjected to detergent exchange, crystallization trials and X-ray structure determination, (iii) to carry out large-scale purification of YedYZ and YdhXU to produce quantities of protein which will be subjected to detergent exchange, crystallization trials and X-ray structure determination and (iv) to develop multidimensional NMR methods to address the structure of heme-containing integral membrane subunits that serve both an anchor and quinol oxidase function.

Grant: 1R01GM068481-01
Program Director: SOMERS, SCOTT D.
Principal Investigator: FINK, MITCHELL P.
Title: Ethyl Pyruvate: A Novel Treatment for Sepsis
Institution: UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PA
PITTSBURGH
Project Period: 2003/07/01-2007/06/29

DESCRIPTION (provided by applicant): Ethyl pyruvate (EP) is the ester formed from pyruvic acid and ethanol. In preliminary studies, we have documented that EP ameliorates intestinal and hepatic injury or improves survival when it is used as a therapeutic agent to treat rodents subjected to mesenteric ischemia and reperfusion, hemorrhagic shock, endotoxemia, or polymicrobial bacterial sepsis. In addition, we have demonstrated that EP is an effective scavenger of reactive oxygen species (ROS), and we have shown that this compound is also an anti-inflammatory agent that inhibits activation of the pro-inflammatory signaling factors, NF-KappaB and p38 mitogen-activated protein kinase. EP also inhibits release of a novel cytokine, high mobility group box 1 (HMGB1). Prompted by these exciting observations, we propose to carry out a series of experiments that are designed to better elucidate the mechanism(s) responsible for the anti-inflammatory and therapeutic effects of EP. The work will be organized under three Specific Aims. Aim 1 is to test three broad hypotheses that might account for the beneficial effects of EP. These hypotheses are: the ROS Hypothesis, the Alkylation Hypothesis, and the Glutathione (GSH) Depletion Hypothesis. The ROS Hypothesis proposes that EP's ability to function as an antioxidant accounts for its cytoprotective and anti-inflammatory effects. The Alkylation Hypothesis proposes that EP functions as an electrophile that alkylates key thiol groups and thereby inactivates important signaling or effector molecules, such as subunits of the transcription factor, NF-KappaB, or various caspases involved in the process of apoptosis. The GSH Depletion Hypothesis, a variant of the Alkylation Hypothesis, proposes that EP alkylates GSH. Depletion of GSH shifts the cellular redox balance in a way that favors the formation of mixed disulfides with NF-KappaB subunits and thereby interferes with signaling via this pathway. Aim 2 is to carry out more detailed studies on the effects of EP on NF-KappaB activation. Aim 3 is to investigate the mechanisms responsible for inhibition of HMGB 1 release by EP. All of these aims will be carried out using a combination of molecular and pharmacological approaches and the studies will employ both in vitro (cell culture) and in vivo (animal model) approaches. Achieving a better understanding of the mechanisms responsible for the anti-inflammatory and therapeutic effects of EP may permit identification of novel cellular pathways involved in the innate immune response.

Grant: 1R01GM068550-01
Program Director: IKEDA, RICHARD A.
Principal Investigator: RAUSHEL, FRANK M. PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: Enzymic Detoxification of Organophosphate Nerve Agents
Institution: TEXAS A&M UNIVERSITY SYSTEM COLLEGE STATION, TX
Project Period: 2003/07/01-2007/06/30

DESCRIPTION (provided by applicant): The long-term objective for the research described in this proposal is the design and construction of a robust catalytic system that can be exploited for the decontamination, destruction, and detection of organophosphate nerve agents. The nerve agents sarin (GB), soman (GD), and VX are among the most toxic and deadly nerve agents ever reported. Since these compounds are easy to synthesize and distribute, they represent a serious threat to the health and well being of civilized societies. In order to prepare a system that can detoxify organophosphate nerve agents, we will manipulate and enhance the enzymatic power of wild type proteins to serve as catalysts for the recognition and hydrolytic turnover of these highly toxic materials. It has been demonstrated that the catalytic machinery embedded within the active sites of the bacterial phosphotriesterase and alpha-prolidase are capable of hydrolyzing certain organophosphate nerve agents such as paraoxon at the diffusion controlled limit. The active site structures of these enzymes will be reengineered through rational and combinatorial mutagenesis techniques to create libraries of mutant enzymes with altered catalytic properties. These enzyme libraries will be efficiently evaluated with high throughput screening protocols using fluorescence and visible spectroscopy with chiral analogs of GB, GD, and VX. The catalytic activities with the restricted nerve agents will be optimized through a direct collaboration with the DeFrank group at the Aberdeen Proving Ground. The structural analyses of the wild type and mutant proteins will be conducted by the Holden group at Wisconsin.

Grant: 1R01GM068585-01
Program Director: CHIN, JEAN
Principal Investigator: MORAIS CABRAL, JOAO H PHD
Title: Structural and functional studies of ion transporters.
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 2003/08/01-2008/07/31

DESCRIPTION (provided by applicant): Ion symporters are membrane proteins that mediate the co-transport of two different ion species across the membrane. They play important roles in human physiology; the Na⁺/I⁻ symporter, for example, is responsible for accumulation of I⁻ in the thyroid gland. Despite their importance a detailed mechanistic understanding of how symporters work is lacking. The long term objective of this proposal is the development of a detailed molecular model for the co-transport of two ions, Na⁺ and K⁺ by KtrAB, a bacterial symporter. The major aims of this proposal are: 1 - Characterize the transport properties of the KtrAB symporter. 2 - Characterize the structure and function of KtrA. 3 - Determine the crystal structure of the membrane protein KtrB.

Grant: 1R01GM068851-01
Program Director: ECKSTRAND, IRENE A.
Principal Investigator: MEKALANOS, JOHN J PHD
Title: Phages and Genomic Variation in V. Cholerae Evolution
Institution: HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA
Project Period: 2003/09/01-2007/08/31

DESCRIPTION (provided by applicant): The goal of this project is to characterize the mechanisms governing the evolution of *Vibrio cholerae* strains with epidemic potential within the context of a natural endemic environment. Emphasis will be placed on molecular genetic analysis of the role of vibriophages (bacterial viruses that grow on *Vibrio* species) in horizontal transfer of virulence-related genes, and in modulating *V. cholerae* population dynamics in the environment. Vibriophages will be isolated from 10 environmental surveillance sites in Bangladesh and characterized with respect to their host range, environmental prevalence, and ability to package and transfer *V. cholerae* DNA encoding virulence-related genes. Any phage that correlates negatively or positively with the presence of specific subgroups of *V. cholerae* will be monitored more closely with specific PCR or nucleic acid probes. In this way we hope to assess the phages ability to modulate the prevalence of specific *V. cholerae* strains, thereby affecting the ability of these strains to cause outbreaks of cholera. Environmental and clinical *V. cholerae* strains will also be collected, analyzed by microarray, DNA sequencing, and genetic fingerprinting methods to establish the phylogenetic relationships among the strains. The virulence gene content of the collected strains will be determined, and variant alleles of certain genes will be tested for their ability to mediate virulence-related functions (e.g., intestinal colonization). Clinical isolates that do not possess known colonization factor genes will be subjected to genetic analysis in order to identify the novel determinants carried by these strains. *V. cholerae* present in cholera stools have been reported to show increased infectivity in infant mice. We will examine this phenomenon using a variety of approaches including physiology assays, expression profiling with *V. cholerae* microarrays and quantitative chemotaxis assays. The results of this project will not only provide a full understanding of the processes by which pathogenic *V. cholerae* strains emerge, evolve, and transmit, but will also guide the development of surveillance and preventive measures that can be used to combat cholera in a cholera endemic area.

Grant: 1R01GM068903-01
Program Director: ANDERSON, JAMES J.
Principal Investigator: HATFIELD, G WESLEY PHD BIOLOGY NEC:BIOL
NEC-UNSPEC
Title: Global Regulatory Networks in Escherichia Coli
Institution: UNIVERSITY OF CALIFORNIA IRVINE IRVINE, CA
Project Period: 2003/09/19-2007/08/31

DESCRIPTION (provided by applicant): We have previously described DNA supercoiling-dependent mechanisms involving protein (IHF or FIS)- mediated translocation of local superhelical energy from one supercoiling-induced duplex destabilized (SIDDD) region on the chromosome to another that serve to coordinate the basal levels of expression of the *itvGMEDA*, *leuV*, and *ilvYC* operons of the *ilv* regulon of *Escherichia coli*, both with one another and with the nutritional and environmental states of the cell. Here we propose to employ computational and biological methods to determine the extent to which these mechanisms are used for the global regulation of gene expression in this model organism. More specifically, we will use methods involving computational prediction and experimental verification to bring together three separate lines of inquiry to determine all the genes of *E. coli* that have the catenation of properties needed for IHF-mediated regulation by a mechanism involving a binding-induced transmission of destabilization. First, we will use computational methods to predict the locations of all the SIDDD sites on the *E. coli* chromosome at superhelical densities encountered in otherwise isogenic wild-type, topoisomerase (*topA*, or *gyrB*) deficient *E. coli* strains. Second, we will develop and apply a novel method to computationally search the *E. coli* genome to identify all high affinity IHF binding sites. Third, we will use DNA microarrays to obtain gene expression profiles in IHF + and IHF- cells at the superhelical densities encountered in these strains. These data will allow us to identify genes (operons) that are regulated either by DNA supercoiling, or by IHF, or both. Genes whose upstream flanks contain a SIDDD site that coincides with or overlaps a strong IHF binding site, and which are shown to have IHF dependent expression will be identified, and subjected to further experimental study to verify: 1) that superhelicity destabilizes the predicted SIDDD site; 2) that IHF binds at the predicted location; 3) that changes in duplex destabilization patterns alone, without changes in the local base sequence, affect gene regulation; and 4) that IHF binding regulates this expression in a DNA-supercoiling-dependent manner in an in vitro system.

Grant: 1R01GM069320-01A1
Program Director: JONES, WARREN
Principal Investigator: ZABRISKIE, T MARK PHD
Title: BIOSYNTHESIS OF ANTITUBERCULAR NONRIBOSOMAL PEPTIDES
Institution: OREGON STATE UNIVERSITY CORVALLIS, OR
Project Period: 2003/09/30-2007/08/31

DESCRIPTION (provided by applicant): Tuberculosis (TB) kills more young and middle-aged adults worldwide than any other disease with the exception of AIDS. Furthermore, TB is the leading cause of morbidity and mortality among the population living with HIV/AIDS. While TB is generally perceived as a disease afflicting the poorest countries, the rapid increase in cases of multidrug-resistant tuberculosis (MDR-TB) and its spread to industrialized nations makes the disease a global threat. As primary drugs are rendered ineffective, second-line agents that are more expensive, usually less effective and often more toxic must be used. Hence, the need for new anti-TB drugs has never been greater, yet it has been over 30 years since the introduction of the last novel antitubercular agent. Advances to improve potency, decrease toxicity, and shorten the duration of therapy would have an enormous impact on world health and healthcare costs. This application describes an approach to access, utilize and manipulate peptide biosynthesis genes that direct precursor construction and post-assembly tailoring to create novel antitubercular peptides. The targets are analogs of viomycin, a peptide antibiotic produced by *Streptomyces vinaceus* and a second-line antitubercular agent that is often effective against MDR-TB. Viomycin possesses rare and unique amino acids that are crucial for biological activity but are not available for chemical synthesis. The proposal describes studies on the biochemical transformations leading to these novel amino acids, including reactions proposed to occur on peptidyl carrier (PCP) protein-bound species. Experiments are proposed to generate new peptides through targeted gene disruption and directed biosynthesis with precursor analogs added exogenously or formed in situ. Novel compounds will also be formed by controlling enzymes that decorate and fully activate the core peptide. Successfully harnessing the genes and deciphering the biochemical mechanisms involved in viomycin precursor assembly and tailoring will yield valuable information that will be translated into novel molecular tools for generating new antitubercular agents. The knowledge and methods that arise from these studies will be directly applicable to expanding the chemical diversity in other families of bioactive peptides.

Grant: 1R01GM071318-01
Program Director: CASSATT, JAMES
Principal Investigator: HANSON, DEBORAH K PHD
Title: Overexpression of human HIV coreceptors in Rhodobacter
Institution: UNIVERSITY OF CHICAGO CHICAGO, IL
Project Period: 2003/09/10-2007/08/31

ABSTRACT NOT PROVIDED

Grant: 1R15GM063514-01A1
Program Director: DEATHERAGE, JAMES F.
Principal Investigator: HALKIDES, CHRISTOPHER J AB
Title: Complexes of CheY in the Active Form with Peptides Deriv
Institution: UNIVERSITY OF NORTH CAROLINA WILIMINGTON, NC
WILMINGTON
Project Period: 2003/03/01-2006/02/28

DESCRIPTION (provided by applicant): This proposal addresses bacterial signal transduction at the molecular level, meaning how bacteria sense and respond to changes in their environment. More specifically the proposed research focusses on chemotaxis, the movement of bacteria up or down a chemical concentration gradient. Bacterial signal transduction depends upon a family of proteins known as response regulators, of which CheY, the response regulator in chemotaxis, is the most well studied. CheY is a signalling protein with an inactive state and a short-lived active state. In its active state CheY interacts with two proteins, CheZ and FliM. Until the recent creation of long-lived analogs of the active state, it had been difficult to study the active state of CheY and other response regulators. Response regulators are a logical target for drug design because mammals do not possess this family of proteins. Specifically, drugs that disrupt chemotaxis in pathogenic bacteria might thwart their pathogenicity. This research will use X-ray crystallography to solve the structures of CheY proteins and will use fluorescence quenching to determine the dissociation constants of peptides derived from CheZ and FliM. Along with the known phenotypes of mutants of CheY, these data will allow us to define which portions of the structure affect the function of CheY. Specifically, this research will test the hypothesis that residue Tyr106 is part of the signalling surface by using the Thr87Ile mutant of CheY. Signalling in this mutant is thought to be impaired because Ile87 forces the rotameric position of Tyr106 into its nonsignalling state. In addition, we will study the Lys109Arg mutant of CheY, which is also impaired in its ability to signal, by the same means. We will co-crystallize active CheY with a peptide derived from CheZ to determine whether or not CheZ promotes the phosphatase activity of CheY by inserting a residue directly into the active site of CheY.

Grant: 1R15GM068690-01
Program Director: IKEDA, RICHARD A.
Principal Investigator: MCCLEARY, WILLIAM R PHD
Title: GENETIC AND BIOCHEMICAL STUDIES OF PHOB ACTIVATION
Institution: BRIGHAM YOUNG UNIVERSITY PROVO, UT
Project Period: 2003/08/01-2006/07/31

DESCRIPTION (provided by applicant): The objective of the proposed research is to understand how signals are transmitted through the PhoB-PhoR two-component signal transduction pathway in *Escherichia coli*. Two-component signal transduction systems control many aspects of the physiology of microorganisms. The phosphate signaling system is present in many bacteria and provides a model for understanding other phosphorylation-based signaling cascades. The phosphate signaling pathway is comprised of a phosphate transporter, PstSCAB; an auxiliary protein of unknown biochemical function, PhoU; the histidine kinase, PhoR, which receives and processes environmental signals; and the response regulator, PhoB, which functions as a transcriptional activator. PhoB is a multi-domain protein whose N-terminal domain becomes phosphorylated on an aspartate residue and whose C-terminal domain binds DNA and interacts with RNA polymerase to activate transcription. The proposed experiments will employ genetic and biochemical techniques to identify amino acid residues that are involved in the propagation of the aspartyl phosphorylation-based signal from the receiver domain to the output domain. In addition, the research will also investigate the 6-7 amino acid linker segment that connects the two domains by conducting site-directed mutagenesis experiments and analyzing the phenotypes of the mutants. Attempts will be made to understand the role of each residue in the linker region. Very little is known about the PhoU protein. Its domain structure will be investigated by using partial proteolysis experiments and through a genetic deletion analysis. PhoU's cellular localization will be determined using GFP-PhoU fusion proteins and/or through immunoelectron microscopy. In addition, the amounts of PhoU, PhoR, PhoB, and the PstSCAB transporter under different growth conditions will be determined through quantitative Western blot analysis. The effects of under- and over-expression of PhoU will also be determined in a phoU minus genetic background. The proposed work is important because these signaling proteins are essential for bacteria to survive changing environments. This feature, combined with their absence in higher eukaryotes, makes them targets for the development of new antibiotics. An increased understanding of these signal transduction proteins will assist in the rational design of drugs to combat pathogens.

Grant: 1R21GM067061-01

Program Director: EDMONDS, CHARLES G.

Principal Investigator: INOUE, MASAYORI PHD
GENETICS:BIOCHEMICAL/M
LECULAR

Title: Cold Shock Vectors for Single Protein Production

Institution: UNIV OF MED/DENT NJ-R W JOHNSON MED PISCATAWAY, NJ
SCH

Project Period: 2003/05/01-2005/04/30

DESCRIPTION (provided by applicant): Is it possible to produce only a single protein of interest in an extremely large amount in *E. coli* so that structural determination of that protein can be carried out without purification? In the present proposal, we will attempt to develop a novel cold-shock-vector-host system that converts *E. coli* cells into such a protein-synthesizing machinery dedicated to the production of only a single protein of interest in a very high yield upon cold shock. For the last 12 years, we have been working on a number of proteins specifically induced at extremely high levels upon temperature downshift in *E. coli*. In particular, the mRNA for CspA, the major cold shock protein in *E. coli* is induced at a very high level, and is translated in a highly efficient manner so that all cellular ribosomes can be trapped with this mRNA. This effect (called the LACE effect) causes complete cell-growth inhibition as a result of almost complete inhibition of all the other cellular proteins. In the present proposal, using the unique features of the cspA promoter and the cspA mRNA, we will attempt to construct cold-shock vectors for exclusive production of a protein of interest in *E. coli*. In order to achieve our goal, the host strain will be improved for further stabilization of the mRNA for the cloned gene, higher translation initiation, and prevention of other cellular protein synthesis upon cold shock. In a preliminary result, few eukaryotic proteins that are either produced very poorly or not produced at all using the conventional T7 expression system can be produced at a very high yield using a prototype cold-shock vector system. With the proposed system, protein production is induced only at low temperatures, therefore unstable proteins will be produced in a better efficiency as compared with other systems. Our goal is to achieve more than 90% efficiency for a target protein synthesis upon cold shock and more than 70 % yield for the cloned gene product with respect to the total cellular proteins. Thus, a cloned gene product can be easily purified. Furthermore, a target protein can be specifically labeled with isotopes such as ¹³-C and ¹⁵-N so that NMR spectroscopy can be carried out directly with cell lysates after simply removing insoluble materials by centrifugation without any further purification. The proposed vector-host system will be highly useful not only for structural biology but also for medicinal research involving human proteins, which can not be obtained by conventional expression systems.

Grant: 2R37GM027750-24
Program Director: CHIN, JEAN
Principal Investigator: SPUDICH, JOHN L
Title: Structure/Function of Microbial Sensory Rhodopsins
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX
HOUSTON
Project Period: 1980/04/01-2008/03/31

DESCRIPTION (provided by applicant): The long-term goal is to elucidate structure/function relationships in the microbial sensory rhodopsins, a family of visual pigment-like heptahelical photoreceptors, to elucidate principles of membrane receptor photoactivation and receptor interactions with membrane-bound and cytoplasmic transducers during signal transduction. Sensory rhodopsins I and II (SRI and SRII) are phototaxis receptors in haloarchaea, each of which couples to its cognate membrane-embedded transducer protein (HtrI and HtrII) to control a phosphorylation cascade that modulates the cell's motility. The SR receptors show close mechanistic similarities in their activation process to higher animal visual pigments, and their presence in a microorganism makes them more amenable to classical and molecular genetic analysis, combined with biochemical and biophysical approaches. In the current grant period the investigators have determined the dark structure of SRII at atomic resolution from two-dimensional (2-D) lipid bilayer crystals and three-dimensional (3-D) cubic lipid phase crystals by cryoelectron image analysis and x-ray diffraction. The applicants plan to use the crystals to obtain atomic resolution structures of SRII photocycle intermediates implicated in signal transfer to its transducer by applying freeze-trapping methods, as well as by crystallography of constitutively activated mutant forms of SRII bound to transducer fragments active in signal transfer. The structural information obtained will complement random mutagenesis, mutant selection, and functional analyses to identify residues critical in receptor activation and transducer signal relay, and will guide site-specific mutagenesis and time-resolved spectroscopy (ultraviolet (UV)-visible and FTIR spectroscopy) to monitor chemical events during the photosignal transduction process. Work over the past three years has revealed a previously unsuspected widespread family of photoactive proteins closely related to the haloarchaeal sensory rhodopsins. Organisms containing these proteins inhabit such diverse environments as soil, freshwater, salt-flats and ocean waters, and human and plant tissues as pathogens (e.g., the fungi *Cryptococcus neoformans* and *Cryptonectria parasitica*), and they comprise a broad phylogenetic range, including haloarchaea, proteobacteria, cyanobacteria, and eucaryotic microbes (fungi and algae). Experiments are designed to elucidate structure/function of selected members of the microbial rhodopsin family to test and expand the "unified mechanism" model for heptahelical protein photosignaling and transport put forth based on the analysis of the haloarchaeal proteins.

Grant: 2R37GM044100-14
Program Director: SOMERS, SCOTT D.
Principal Investigator: BILLIAR, TIMOTHY R
Title: Nitric Oxide and Hepatic Function in Sepsis and Trauma
Institution: UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PA
PITTSBURGH
Project Period: 1990/04/01-2008/06/30

DESCRIPTION (provided by applicant): Nitric oxide (NO) and its reaction products are key players in the physiology and pathophysiology of inflammatory settings such as sepsis and shock. Since originally funded in 1989, research supported by this grant has led to the following advances: the initial characterization of NO, production by liver cells, the molecular identification of iNOS expression by hepatocytes, the initial proof that humans express iNOS, and the first cloning of the human iNOS cDNA and gene. This work established the foundation for studies characterizing the regulation of human iNOS expression in hepatocytes at the transcriptional, post-translational, and substrate levels. The consequences of the expression of inducible NO synthase (iNOS, NOS-2) can be either protective or damaging to the liver. More recently, we have delineated two distinct hepatoprotective actions of NO.: the stimulation of cyclic guanosine monophosphate (cGMP) and the inhibition of caspases by S-nitrosation. In contrast, iNOS/NO, promotes hepatocyte death under conditions of severe redox stress, such as hemorrhagic shock or ischemia/reperfusion. Redox stress activates an unknown molecular switch that transforms NO, which is hepatoprotective under resting conditions, into an agent that induces hepatocyte death. We hypothesize that the magnitude of the redox stress is a major determinant for the effects of NO, on cell survival by controlling the chemical fate of NO. We propose to test this hypothesis in two interrelated aims: I) to determine how cellular redox status regulates the chemical fate and function of NO in hepatocytes, and II) to determine how cyclic nucleotides (cGMP, cAMP) prevent hepatocyte apoptosis. The hypothesis to be tested under Aim I is that different redox reaction products of NO are produced under settings of redox stress vs. non-stress conditions, and these reaction products of NO are either themselves toxic or reduce the anti-apoptotic efficacy of NO. Aim I will be pursued through in vitro work in hepatocytes exposed to either normoxic or hypoxic oxidant stress, and examining the reaction products of NO, that are formed. In vivo work in a model of hepatic ischemia/reperfusion will serve to confirm selected aspects of this work. The hypothesis to be tested under Aim II is that the protective actions of cyclic nucleotides in hepatocytes are mediated through the regulation of the pro-apoptotic molecule FADD via protective pathways that involve cyclic nucleotides and protein kinase A. Aim II will be pursued through delineation of the level at which FADD is modulated during apoptosis by cyclic nucleotides, and through the use of pharmacological inhibitors of relevant pathways. These studies will comprehensively elucidate how the redox state in hepatocytes determines the chemical fate and function of NOx as well as the downstream effects of NOx and cyclic nucleotides on the homeostatic mechanisms that control hepatocyte survival and death.

Grant: 2R37GM044118-12
Program Director: SOMERS, SCOTT D.
Principal Investigator: HOTCHKISS, RICHARD S MD ANESTHESIOLOGY
Title: Prevention of Apoptotic Cell Death in Sepsis by BCL-2
Institution: WASHINGTON UNIVERSITY ST LOUIS, MO
Project Period: 1999/08/01-2008/07/31

DESCRIPTION (provided by applicant): Sepsis is the leading cause of death in most intensive care units with over 210,000 people succumbing to overwhelming infection (or the resultant multiple organ failure) in the United States annually. A recent epidemiologic study estimated that 750,000 people develop sepsis annually at a cost of \$16.7 billion dollars. Studies show that apoptosis is an important mechanism of cell death in sepsis and that prevention of apoptosis can improve survival in animal models of sepsis. Apoptosis can proceed by either a receptor or mitochondrial mediated pathway. In certain types of cells subjected to particular apoptotic stimuli, the two pathways can be linked such that both pathways are operative in the same cell. Understanding the mechanisms of apoptotic cell death in sepsis is vital because this knowledge may reveal the inciting stimuli and enable an effective therapy aimed at the responsible pathway. Currently, the particular apoptotic pathway operative in sepsis, i.e., receptor versus mitochondrial, is debated and both pathways have been reported to be activated. Preliminary findings in our laboratory indicate that both pathways may be operative in sepsis-induced lymphocyte apoptosis although they appear to be occurring in anatomically different regions of the spleen, consistent with effects on different lymphocyte phenotypes. Like Bcl-2, the serine threonine kinase Akt is an oncoprotein which prevents cell death due to a variety of apoptotic stimuli. The exact mechanism of action of Akt is unknown but in some instances it appears to work by a mechanism that is distinct from that of Bcl-2. Preliminary findings in our laboratory show that lymphocytes from mice that over express Akt in T cells do not undergo sepsis-induced apoptosis and the mice have improved survival compared to non-transgenic. The guiding hypotheses of this proposal are: I) lymphocyte apoptosis in sepsis proceeds via both receptor and mitochondrial mediated pathways and, ii) over-expression of Akt in T cells will prevent lymphocyte apoptosis and improve sepsis survival. Studies will be conducted using a clinically relevant animal model of sepsis. In addition, translational studies will be conducted in blood from critically ill patients with and without sepsis. Note that preliminary findings demonstrate increased lymphocyte apoptosis in patients with sepsis versus critically ill non-septic patients. This proposal will provide important new information regarding mechanisms of apoptotic lymphocyte death in sepsis and may ultimately lead to a more effective therapy for this highly lethal disorder.

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Grant: 2R37GM047958-10
Program Director: IKEDA, RICHARD A.
Principal Investigator: STOCK, ANN M PHD
Title: Structure and Function of Response Regulator Proteins
Institution: UNIV OF MED/DENT NJ-R W JOHNSON MED PISCATAWAY, NJ
SCH
Project Period: 1992/08/01-2008/06/30

DESCRIPTION (provided by applicant): A large fraction of bacterial signaling is mediated by "two-component" systems that utilize a conserved mechanism of phosphotransfer between a sensor histidine protein kinase and a response regulator protein. Numerous two-component systems are present in a single bacterium and often function in pathways that are important or essential for pathogenesis. The absence of two-component proteins from animals has made them attractive targets for pursuit of new antimicrobial agents. The conserved domain of response regulators functions as a phosphorylation-activated switch, controlling the activity of the effector domain and the output response. This application focuses on structural and biochemical characterization of the mechanism of activation of response regulator proteins. In particular, the project seeks to determine the relative regulatory contributions of intramolecular interactions between the regulatory and effector domains versus intermolecular interactions between the regulatory domains within functional dimers of the large OmpR/PhoB subfamily of response regulators that function as transcription factors. The studies will involve construction and characterization of chimeric response regulator proteins, determination of surfaces of interaction within active dimers, and structural characterization by X-ray crystallography. The applicant's studies are aimed at providing a detailed description of the regulatory mechanisms of a small number of representative response regulators, with the expectation of significance not only to an understanding of these individual proteins, but also to establishing the similarities and differences within a large family of signaling proteins. This system provides an opportunity to address the extent to which sequence and structural similarity can be used to predict similarity in mechanism of function, an important question that is emerging in this era of proteomics. Additional studies are focused on components of bacterial chemotaxis systems that contribute to receptor-mediated adaptation, specifically studies of regulatory interactions in the multi-domain response regulator methylesterase CheB and structural characterization of proteins CheD and CheC.

Grant: 2P01HD013021-26
Program Director: GRAVE, GILMAN D.
Principal Investigator: MORROW, ARDYTHE L PHD
Title: The Role of Human Milk in Infant Nutrition and Health
Institution: CHILDREN'S HOSPITAL MED CTR CINCINNATI, OH
(CINCINNATI)
Project Period: 1979/07/01-2008/03/31

This application for competitive renewal is submitted by a multi-disciplinary team of senior investigators who propose to continue their studies on bioactive factors in human milk. Our program project is unique in its focus on human milk and the mechanisms by which human milk protects infants against infectious disease. The respective projects of this renewal consider the role of human milk in protection against human caliciviruses; rotavirus; campylobacter and related pathogens; enteropathogenic E. coli and related pathogens; and stable toxin (ST) of E. coli. The project protocols utilize in vitro assays, animal models, and human subjects, and are supported by interaction with cores that address epidemiology, biostatistics and translational research; glycobiology and synthetic chemistry; and molecular biology. In this application for renewal, we propose to examine protection against multiple pathogens through fundamental mechanisms of innate and adaptive immunity that are expressed in human milk, specifically, oligosaccharides and related glycoconjugates, and secretory antibody. This overall theme will be addressed by studying the extent to which these human milk factors inhibit pathogen-cell surface binding and provide cross-protection against multiple pathogens. We also will examine the genetic basis for variability in expression of oligosaccharide protective factors in human milk and infant susceptibility to diarrheal diseases. Based on results of studies conducted in the current grant cycle, this renewal will involve synthesis of oligosaccharides found in human milk and translational research that tests their protective efficacy in pre-clinical studies that lead to carefully designed phase I and II trials in young children.

Grant: 2R01HD030773-11
Program Director: DE PAOLO, LOUIS V
Principal Investigator: KARSCH, FRED J PHD
Title: Neuroendocrine Regulation of the Estrous Cycle
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 1993/09/01-2008/07/31

DESCRIPTION (provided by applicant): The negative impact of stress on ovarian cyclicity and fertility is a significant problem in fertility regulation and women's reproductive health. The overall goal of this proposal is to gain a better understanding of how stress disrupts the cycle and reduces fertility. A common neuroendocrine response to stress is the suppression of gonadotropin secretion (most notably pulsatile LH secretion) coupled to activation of the hypothalamic-pituitary-adrenal axis and enhanced glucocorticoid secretion. Recent findings indicate that, in the absence of stress, an acute stress-like increase in circulating cortisol suppresses pulsatile luteinizing hormone (LH) secretion in sheep. Further, the follicular phase of the cycle and preovulatory LH surge in this species are disrupted by stress-like increments in circulating cortisol. These findings lead to the hypothesis that a stress-like increase in cortisol disrupts the follicular phase by acting at the hypothalamus and pituitary gland to suppress the pulsatile secretion of gonadotropin-releasing hormone (GnRH) and LH necessary for stimulating the preovulatory increase in estradiol secretion and, in turn, the GnRH and LH surges. It is proposed that these actions of cortisol contribute to ovarian cycle disruption during stress. The proposed research tests this hypothesis utilizing the sheep as an animal model and four Specific Aims. First, it will be determined if a stress-like increment in cortisol inhibits hypothalamic GnRH secretion and/or responsiveness of the pituitary gland to GnRH. Second, the neuroendocrine processes through which cortisol acts to disrupt the follicular phase and the impact of this disruption on fertility will be assessed. Third, the possibility that cortisol mediates ovarian cycle disruption in response to certain types of stress will be investigated. Fourth, the relevant receptor(s) that mediate the suppressive actions of cortisol will be identified. Collectively, the research in these four Specific Aims form a comprehensive and cohesive integrated approach to address the mode of action and physiological significance of cortisol in mediating stress-induced suppression of the ovarian cycle and fertility.

Grant: 1R03HD042112-01A1
Program Director: KAUFMAN, STEVEN
Principal Investigator: SCHWEBKE, JANE R MD
Title: Concordance Rates of Mobiluncus Among Sexual Partners
Institution: UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL
BIRMINGHAM
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): Bacterial vaginosis (BV) is the most prevalent form of vaginitis worldwide and has been linked to complications such as preterm birth and STD/HIV acquisition. The etiology of BV remains unknown although its epidemiology mirrors that of a sexually transmitted disease. Most of the microorganisms associated with BV are detected by culture techniques in the healthy vagina in small numbers with the exception of *Mobiluncus* spp. which seems to be strongly associated with BV. However, this organism is a highly fastidious anaerobe and culture may underestimate the true prevalence in vaginal samples. We propose to use polymerase chain techniques (PCR) to examine the concordance rates of *Mobiluncus* species among sexual partners. Our hypothesis is that *Mobiluncus curtisii* is important in the pathogenesis of BV and high concordance rates among sexual partners will be found suggesting sexual transmission.

Grant: 1R03HD043857-01
Program Director: RAJU, TONSE N.
Principal Investigator: CLANCY, KATHRYN A BS
Title: Characterization of iron transport in GBS
Institution: CHILDREN'S HOSPITAL AND REG MEDICAL SEATTLE, WA
CTR
Project Period: 2003/04/01-2005/03/31

DESCRIPTION (provided by applicant): Group B streptococcus is the predominant etiological agent of neonatal bacteremia, sepsis and meningitis, and has been correlated with preterm membrane rupture and premature birth. While a limited number of GBS virulence determinants have been identified, the contribution of iron and iron acquisition to the pathogenesis of GBS infection is unknown. All pathogens require iron and a correlation between bacterial virulence and iron acquisition has been established. However, as the concentration of available iron in the human host (10^{-18} M) is well below that required by bacteria (10^{-8} M), successful pathogens express specific systems to acquire sufficient concentrations of intracellular iron. One such system involved in siderophore-mediated iron uptake, is the focus of this application. Siderophores are high-affinity iron chelators secreted from the bacterial cell to scavenge iron from host iron-binding proteins. Specialized uptake systems transport the siderophore-iron complex across the bacterial membrane. We have identified a putative siderophore-mediated iron transport system, the fhu operon, in GBS. The operon is comprised of four genes, fhuC, fhuD, fhuB, and fhuG, encoding a putative ATP-hydrolysis protein, siderophore (ferrichrome)- iron receptor protein, and two permeases, respectively. In this application, the role of the fhu operon in iron acquisition by GBS will be examined. Basic information on the requirement of GBS for iron, the ability of GBS to utilize siderophores as an iron source, and whether the organism secretes siderophores to acquire iron will first be established. The biochemical characterization of an isogenic mutant strain deficient for fhu will examine the role of this operon in GBS siderophore-iron transport. In order to define the siderophore specificity of the fhu operon, the construction and biochemical characterization of isogenic mutant strains deficient for each gene is proposed. In addition, heterologous expression studies will provide further evidence for the role of the fhu-encoded proteins in siderophore-iron transport. These studies will provide a basic understanding of the molecular mechanism of iron acquisition in GBS and will allow us to design appropriate in vivo studies to determine the role of iron transport in GBS pathogenesis. The potential of identifying novel therapeutic targets to prevent or treat infections is also proposed.

Grant: 1R21HD044861-01
Program Director: LOCK, ALLAN
Principal Investigator: BALLARD, JIMMY D PHD
Title: Impact of Anthrax Toxin on Embryonic Development
Institution: UNIVERSITY OF OKLAHOMA NORMAN NORMAN, OK
Project Period: 2003/08/01-2004/08/14

DESCRIPTION (provided by applicant): *Bacillus anthracis* lethal toxin and edema toxin modify the physiology of cells by disrupting MAPKK signaling pathways and causing accumulation of cAMP respectively. Both MAPKK signaling and cAMP are important regulators of development and exposure of embryos to anthrax toxin could lead to defects. In recent DNA array studies we have found that Wnt signalling may also be disrupted by lethal toxin. Wnt signaling is a major part of development and aberrations in this pathway could also lead to defects in the developing embryo. In light of these observations we carried out pilot experiments on zebrafish embryos and found noticeable defects after treatment with anthrax toxin. These results strongly suggest a better understanding of anthrax toxin's impact on development is needed. In the case of a bioterrorist disseminating *B. anthracis* spores over a populated area, at least 1.5% of the human population will be pregnant and embryos could be exposed to the toxin. Therefore, in order to better understand the impact of anthrax toxin on embryonic development, we will address two specific aims. Specific Aim 1. We will determine the impact lethal toxin and edema toxin have on zebrafish embryo development. Specific Aim 2. We will determine the impact inactive anthrax toxin mutants have on zebrafish embryo development. Specific aim 2 will address the possibility that vaccine and therapeutic candidates might cause developmental defects.

Grant: 2P01HL057345-06

Program Director: LINK, REBECCA

Principal Investigator: VARKI, AJIT P MD OTH GM/BIO

SC:MULTIDISCIPLINARY N

Title: Genetic Modulation of Blood and Vascular Glycosylation

Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA

Project Period: 1997/09/30-2007/11/30

Carbohydrate chains (glycans) are major components of cells and tissues, with a complexity and mass rivaling nucleic acids and proteins. This program focuses on the two major classes of anionic glycans found at the outermost aspects of the cell surface glycocalyx - the sialic acids (Sias) and the glycosaminoglycan (GAG) chains of heparan and dermatan sulfate proteoglycans. The structures of the N- and O-linked glycans of blood cells and plasma glycoproteins are among the best described to date. Specific glycan-binding proteins differentially recognize Sias on these chains, including the selectins (on leukocytes, platelets and endothelium) and the Siglecs (I-type lectins with cytosolic signaling motifs, found on specific blood cell types). Some 13-galactoside-specific lectins can also detect the absence of Sias. Changes in the sialylation of some proteins involved in hemostasis and thrombosis can alter their turnover and function. The GAG chains of the heparan and dermatan sulfate proteoglycans are involved in regulating processes such as blood coagulation, growth factor modulation, endothelial biology, wound repair and leukocyte migration. Most of the physiologic and pathological roles of Sias and GAGs are not evident in cultured cells, but must be explored in the intact organism - and this complexity of mammalian glycans is not fully represented in model invertebrates. On the other hand, relatively few human glycosylation defects in these molecules are known. Therefore, the central theme of this proposal is state-of-the-art genetic manipulation of Sias, GAG chains, and some of their cognate binding proteins in the intact mouse. When systemic gene inactivation models are non-viable or have confusing phenotypes, we will selectively inactivate mouse genes in a cell type-specific and developmentally-regulated manner. Replacement of wild type alleles with recombinant alleles carrying loxp target sites at innocuous positions allows cell-type specific gene eviction, by mating with mice transgenic for Cre recombinase constructs driven by specific transcriptional control sequences. This will also allow a specific focus on glycans of blood cells, endothelium and plasma proteins. We have assembled the necessary expertise to fully analyze the consequences of these genetic manipulations on the structure of hematopoietic and vascular tissues, the structure of the glycans, and the functional consequences to hemostasis, vascular function, angiogenesis, hematopoiesis, inflammation, the innate immune response to infections, and wound healing. These studies are expected to reveal many important functions for these glycans in health and disease.

Grant: 2R01HL016101-30
Program Director: RABADAN-DIEHL, CRISTINA
Principal Investigator: GENNIS, ROBERT B PHD
Title: PROTEIN/LIPID INTERACTIONS
Institution: UNIVERSITY OF ILLINOIS URBANA- CHAMPAIGN, IL
CHAMPAIGN
Project Period: 1988/01/01-2007/11/30

DESCRIPTION (provided by applicant): The proposed research is focused on the catalytic mechanisms and structures of respiratory oxidases. These membrane enzymes catalyze the reduction of O₂ to H₂O, and conserve the considerable free energy liberated by this reaction as a proton motive force. All respiratory oxidases belong to either the proton pumping "heme-copper oxidase" superfamily or the nonpumping "tri-heme oxidase" superfamily. The heme-copper oxidases include all the mitochondrial cytochrome oxidases. As a crucial enzyme in cellular bioenergetics, human cytochrome oxidase is of medical significance, and has been studied as the locus of a set of genetic diseases. The prokaryotic enzyme we are studying from *Rhodobacter sphaeroides* is an excellent model system for understanding how this enzyme functions. The cbb3-type oxidase from *Vibrio cholerae* is a second member of the heme-copper oxidase superfamily that will be studied. Learning how the same functions are accomplished in these two distantly related enzymes should be revealing. Interest in cytochrome oxidase research is primarily directed towards understanding the proton pump mechanism. For each O₂ reduced to H₂O, eight charges are driven across the membrane, generating a substantial transmembrane voltage. Research on the aa3-type oxidase from *R. sphaeroides* utilizes site-directed mutagenesis in combination with a several spectroscopic techniques, including solid state NMR and FTIR difference spectroscopy. The X-ray structure of this enzyme is known and serves as a guide to planning and interpreting experiments. One mutant of particular interest is N139D. This mutation results in increasing the cytochrome oxidase activity of the enzyme by at least 50% over the wild type, but completely eliminates proton pumping. Proton uptake, proton release and intra-protein proton movements will be examined. Understanding how the proton pump is decoupled from the catalytic function will provide fundamental information about how the proton pump works. The tri-heme oxidases are unique to prokaryotes, but are also of medical significance as potential drug targets because of their apparent importance for the virulence of some pathogenic bacteria. Research on the tri-heme oxidases will focus on the cytochrome bd from *E. coli* and the cytochrome bb' from *V. cholerae*. The bb'-type enzyme has just recently been discovered and part of the research plan is to purify and characterize this enzyme for the first time. The tri-heme oxidases have two closely interacting hemes at the active site, and spectroscopic methods will be used to elucidate the details of the heme-heme interaction. Identifying residues involved in intra-protein proton movement from the bulk solution to the active site will also be a goal in the near future. Finally, it is essential to obtain a structure of this enzyme by X-ray crystallography, and efforts will be made to accomplish this.

Grant: 2R01HL030923-21
Program Director: GAIL, DOROTHY
Principal Investigator: WRIGHT, JO RAE PHD OTHER AREAS
Title: Alveolar Factors in Uptake of Lung Surfactant
Institution: DUKE UNIVERSITY DURHAM, NC
Project Period: 1994/07/01-2007/06/30

DESCRIPTION (provided by applicant): Pulmonary surfactant plays important roles in reducing surface tension at the air-liquid interface of the lung and in regulating lung host defense. In order to carry out these roles, a functional pool of adequate surfactant must be maintained by balancing the rates of secretion and clearance. Studies from our and other laboratories have shown that clearance in the normal lung occurs via reuptake and recycling by type II cells, and via degradation by both type II cells and macrophages. Recently we have focused on understanding the factors that affect surfactant metabolism in the injured or inflamed lung and have discerned that inflammatory cells and bacterial products contribute significantly to surfactant degradation. Our recent preliminary data show that *Pseudomonas aeruginosa*, an important pulmonary pathogen, degrades SP-A and SP-D and we have identified enzymes, including *Pseudomonas* elastase, that contribute to this process. In addition, our preliminary data suggest that neutrophils and activated macrophages contribute substantially to surfactant degradation. The hypothesis to be tested in this competitive renewal is that infection and inflammation result in release of degradative enzymes from bacteria and from newly recruited inflammatory cells that result in enhanced degradation and decreases in surfactant pool size. The decrease in the pool of functional surfactant leads to altered lung homeostasis, including decreased lung compliance and increased susceptibility to infection and inflammation. Five specific aims are proposed to test this hypothesis. Specific Aim 1 is to determine if bacterial enzymes degrade surfactant lipids and proteins in vitro. Specific Aim 2 is to determine the role of a newly described *P. aeruginosa* enzyme, Protease IV, in degrading surfactant. Specific Aim 3 is to investigate the functional consequences of degradation of surfactant by bacterial enzymes in vitro. Specific Aim 4 is to determine if surfactant is degraded in vivo. Specific Aim 5 is to investigate the role of activated macrophages and neutrophils in the degradation of surfactant. Results from these studies will help determine if these mechanisms contribute to the alterations in surfactant pool size that are observed patients with acute lung injury. We propose that degradation of surfactant by bacterial enzymes and cells recruited in response to bacterial infection represent a novel mechanism of pathogen adaptation and manipulation of the host response which would contribute to alterations in surfactant pool size and resulting lung injury. Significance: An understanding of the factors that regulate surfactant metabolism in lung injury and infection should contribute to development of therapies targeted at inhibiting surfactant degradation for treatment of surfactant deficiency of diseases such as ARDS and bacterial pneumonia.

Grant: 2R01HL051987-09
Program Director: MASSICOT-FISHER, JUDITH
Principal Investigator: DUNNY, GARY M PHD MICROBIOLOGY, OTTUMWA
Title: Cell Wall Components' Role in Enterococcal Endocarditis
Institution: UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN
Project Period: 1994/12/01-2007/07/31

Enterococcus faecalis can cause a severe form of endocarditis with a high fatality rate. Treatment of this disease is complicated by the high level of intrinsic and acquired resistance of this organism to antibiotics. A plasmid-encoded surface protein called Asc10 mediates formation of bacterial aggregates, in which plasmid transfer occurs at high frequency. Asc10 enhances the virulence of *E. faecalis* in experimental endocarditis, and plasmids encoding this type of protein are common in clinical isolates. Expression of Asc10 in the mammalian host is induced by a host factor. Analysis of the structure/function relationships in this protein have shown that the functional domain involved in bacterial aggregation also mediates virulence properties associated with the protein. The proposed experiments seek to define the mechanisms by which specific molecular interactions between Asc10 and host components contribute to the disease process. Analysis of gene expression profiles of the host and pathogen during infections will be carried out. The specific aims include: 1) Determine the specific biological activities of Asc10 affecting *E. faecalis* virulence. 2) Determine the functional domains of Asc10 that confer the activities identified in Aim 1. 3) Examine the expression of bacterial and host genes during the course of infections.

Grant: 2R01HL061407-05
Program Director: HARABIN, ANDREA L.
Principal Investigator: WORTHEN, G S MD INTERNAL
MED:PULMONARY DISEAS
Title: LPS-induced Neutrophil Accumulation in the Lung
Institution: NATIONAL JEWISH MEDICAL & RES CTR DENVER, CO
Project Period: 1998/12/07-2007/11/30

DESCRIPTION (provided by applicant): Cells react to microorganisms by activating innate immune responses that rely on recognition of non-self molecular patterns, such as the bacterial cell wall component lipopolysaccharide (LPS). Exposure of the adherent neutrophil to the LPS component of gram negative bacteria results in an integrated response involving activation of JNK MAP kinase, which is involved in a variety of downstream functional effects that may contribute to the pathogenesis of ARDS. We propose that the adherent neutrophil assembles a signaling complex involving activation of tyrosine kinase Syk, and utilizes adaptor molecules, particularly SLP-76, previously known in mediating signal transduction in the lymphocyte, but never previously implicated in neutrophil signal transduction. We further suggest that the JNK pathway is regulated not only in the activation of membrane proximal adaptors, but is also influenced by cross-talk between p38 MAP kinase and JNK. We suggest that p38 activates PP2A, a phosphatase that inactivates MKK4. Using a variety of techniques to detect molecular interactions and modify expression levels in human and murine neutrophils, and transfectable neutrophilic cell lines we will address 2 major specific aims: 1) To define the molecular mechanisms by which the LPS receptor complex activates JNK in the adherent neutrophil, and 2) To determine the mechanisms by which p38 regulates activation of the JNK pathway. The results of these investigations will highlight novel aspects of neutrophil signal transduction in response to LPS that lead to activation of JNK, and provide insights into the assembly of multi-component signaling complexes whose interactions will provide new approaches to modulation.

Grant: 2R01HL062608-11

Program Director: PEAVY, HANNAH H

Principal Investigator: VASIL, MICHAEL L PHD
MICROBIOLOGY:BACTERIOLOGY

Title: Novel Class of Phospholipases-Molecular Pathogenesis

Institution: UNIVERSITY OF COLORADO DENVER/HSC DENVER, CO
AURORA

Project Period: 1993/06/01-2008/08/31

DESCRIPTION (provided by applicant): The diversity of roles that phospholipases C (PLCs) play in biology and medicine is extraordinary. In the past decade this class of phospholipid hydrolyzing enzymes has been shown to be considerably more complex than initially perceived and their impact on a wide range of basic cellular processes in eukaryotes, including oncogenesis, apoptosis, and inflammation has been increasingly appreciated. Likewise, there are many sundry and important functions for PLCs in microbial pathogenesis. We identified and characterized the first member of a novel class of homologous PLCs, the hemolytic phospholipase C (PIcH) of *Pseudomonas aeruginosa*. Members of this class of PLCs are produced by an array of opportunistic and frank pathogens, including potential bioterrorist agents. The genomes of some of these organisms encode as many as 4 homologs of this class of PLCs. Bacteria carrying genes encoding these PLCs (gene copy number shown in parentheses) include: *P. aeruginosa* (2), *Mycobacterium tuberculosis* (4), *Francisella tularensis* (1), *Burkholderia pseudomallei* and *mallei* (3 each) and *Bordetella pertussis* (1). We, as well as others, provided cogent evidence that members of this novel class of PLCs play significant and diverse roles in the infectious diseases caused by those agents. Although these PLCs share considerable amino acid homology, each member has distinct properties. There are some important differences in their substrate specificities, and many members have unique structural features that probably play a specific functional role in the pathogenesis of the organisms that produce them. This application will mainly focus on the paradigm of this novel class of PLCs (PIcH). In addition to its PLC activity, PIcH is the first prokaryotic or eukaryotic protein yet identified that has Sphingomyelin Synthase activity. The substrates (e.g. phosphatidylcholine & sphingomyelin) of PIcH or the products (e.g. diacylglycerol, ceramide or sphingomyelin) that it generates could have profound biological effects, particularly with respect to signaling processes in eukaryotic cells and the host responses to this infectious agent. We have also provided evidence that PIcH is highly cytotoxic for endothelial cells and probably enters these cells through interaction with integrin receptors. This research project will employ microbiological, genetic, biochemical, structural and cell biology methods to examine how PIcH and other members of this novel class of enzymes affect the virulence of the organisms that produce them. Furthermore, it is likely that information we derive from our efforts will also provide additional insights about the biochemistry and biology of PLCs in general.

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Grant: 1R01HL067971-01A2
Program Director: LIANG, ISABELLA
Principal Investigator: MESTRIL, RUBEN PHD
Title: Cardioprotection against endotoxins
Institution: LOYOLA UNIVERSITY CHICAGO MAYWOOD, IL
Project Period: 2003/07/01-2007/06/30

DESCRIPTION (provided by applicant): A number of studies have shown that rodents submitted to a mild whole body heat shock become resistant to a subsequent lethal dose of lipopolysaccharides (LPS). It is still unclear how a pre-heat treatment protects against endotoxins but one of the main results of a heat shock is the increased synthesis of a group of proteins known as the heat shock proteins (hsp). The hsp70 is the most abundant of these hsps and has been postulated to be the principal mediator of the observed protection against LPS. Since one of the major consequences of endotoxemia is the induction of myocardial contractile dysfunction and hsp70 has been previously shown to protect against myocardial dysfunction due to infarction. We therefore propose to investigate the mechanism by which the hsp70 protects the myocardium during endotoxemia. Our preliminary results show that heat shocked isolated rat neonatal cardiomyocytes are tolerant to a subsequent exposure to LPS. In addition, cardiomyocytes infected with adenoviral vectors containing the hsp70 gene are also tolerant to endotoxin exposure in vitro. We also find that a transgenic mouse line overexpressing a hsp70 gene is more tolerant to endotoxemia than littermates that do not express the hsp70 transgene. The mechanism in which hsp70 renders the cardiomyocyte both in vitro and in vivo tolerant to endotoxemic injury would seem to involve a reduction in inducible nitric oxide synthase expression according to our preliminary results. We therefore propose to study in both isolated cardiomyocytes and the heart tissue of transgenic mice, the level of the mediators of endotoxin-induced cell injury: TNF-alpha, IL-1, nitric oxide (NO), inducible NO synthase and the transcription factor NFkB and how they are affected by the increased presence of hsp70. The proposed research project should clearly demonstrate if the sole presence of increased levels of hsp70 is protective against endotoxemia. This proposed study may open the way to harness the endogenous protective mechanisms in cardiomyocytes and to new innovative and effective therapies against the myocardial depression caused by endotoxins.

Grant: 1R01HL068595-01A2
Program Director: PEAVY, HANNAH H
Principal Investigator: HOVELL, MELBOURNE F MA PSYCHOLOGY
Title: Promoting Adherence to TB Regimens in High-Risk Youth
Institution: SAN DIEGO STATE UNIVERSITY SAN DIEGO, CA
Project Period: 2003/09/15-2008/08/31

DESCRIPTION (provided by applicant): Tuberculosis (TB) was responsible for almost one billion deaths in the 20th century. It is epidemic in the developing world and immigrants introduce TB to developed nations. TB control requires treatment for LTBI and active disease, as well as adherence to medical regimens. We propose to determine the effectiveness of a public health model of LTBI control among high-risk adolescents. The integration of behavioral science, medical services, parent instruction, assistance from schools and clinics, all coordinated by the County Health Department, is based on recommendations from CDC. The effectiveness of this system is dependent, in part, on patients' adherence. The proposed study will demonstrate a public health program for TB control among PPD+ adolescents and conduct a controlled trial of peer counseling plus parent education for adherence to INH. We will test these procedures with high-risk adolescents, from Latino, Asian, and foreign-born populations, for whom latent TB is epidemic. High school students will be screened and 300 PPD+ male and female youth (13-18 yrs.) will be assigned at random to either usual medical care plus non-directed (attention control) counseling, or to usual medical care plus peer adherence counseling and parent instructions to support adherence. Parents of eligible youth will be provided instruction about TB, their child's risk, and preventive treatment. Parents of youth assigned to the peer counseling condition will obtain additional instruction to use contingency management to support their child's adherence to INH. Clinic personnel will receive continuing medical education (CME) related to TB. The primary specific aims are: 1) to determine whether peer counseling increases adherence to prescribed INH medication relative to controls and 2) to determine the cost and cost effectiveness of the intervention. We will determine if training increases parents' and professionals' knowledge of TB control and we will explore possible determinants of adherence to INH. Reported adherence (number of pills taken/month), pill counts and electronic dispenser measures will be verified by monthly impromptu urine assays. Multivariate statistical tests will be used to determine possible mediators/moderators and other predictors of adherence over 12 months. The assessment of both outcomes and relative costs will inform the effectiveness and practicality of a larger scale TB prevention program for high-risk youth in San Diego and other regions where TB is epidemic.

Grant: 1R01HL070231-01A1
Program Director: TOLUNAY, ESER
Principal Investigator: MCINTYRE, THOMAS M PHD BIOCHEMISTRY, OTH
Title: Endotoxin Generated Lipid Second Messengers
Institution: UNIVERSITY OF UTAH SALT LAKE CITY, UT
Project Period: 2002/12/05-2006/11/30

DESCRIPTION (provided by applicant): The events that initiate and promote atherogenesis are not well defined, but sequestration of LDL in the vascular wall at sites prone to lesion development is one. Bound LDL oxidizes to a pro-atherogenic form that is bound by scavenger receptors like CD36, leading to inappropriate intracellular lipid accumulation and foam cell formation. Atherosclerotic lesions can contain Chlamydia pneumoniae, and such infections constitute a risk factor for atherosclerosis. Macrophage infection by C. pneumoniae results in foam cell formation, as does exposure to its purified lipopolysaccharide (LPS). How LPS induces lipid accumulation or stimulates gene transcription is unknown. We find that LPS induces expression of the CD36 scavenger receptor gene in monocytes, leading to enhanced surface expression, and to intracellular lipid droplet accumulation and foam cell formation. CD36 is controlled by the transcription factor PPARgamma, and we find that LPS, unexpectedly, activates a PPAR responsive element (PPRE)-reporter. In fact, LPS activates the CD36 promoter through its PPRE. PPARgamma is activated by lipid ligands; synthetic drugs do this, but high affinity physiologic PPAR3, ligands are unknown. LPS does not bind PPARgamma, so it induced the formation of an endogenous agonist. We find that lysophosphatidic acid (LPA) is a PPARgamma ligand and agonist --providing the first evidence that this might be the long sought after physiologic agonist for this transcription factor. LPA stimulates PPRE-driven reporters, induces CD36 expression, and differentiates monocytes to foam cells. We show this signaling is independent of Edg (surface LPA receptors) signaling in several ways. We find that LPS increases cellular LPA levels, and that metabolizing this intracellular LPA by transfected LPA acyltransferase blocks PPAR7 activation and function. Here we propose to define the way in which LPS induces LPA accumulation, and determine the consequences of LPA activation of an intracellular nuclear hormone receptor/transcription factor on foam cell formation. Knowing the identity of the physiologic ligand has allowed us to establish a high throughput screen for rationally designed PPARgamma inhibitors. One non-hydrolyzable LPA analog blocks PPARy function and might define a new class of anti-inflammatory, anti-lipidic agents.

Grant: 1R01HL070293-01A1
Program Director: CROXTON, THOMAS
Principal Investigator: LI, JIAN-DONG MD
Title: Regulation of Toll-like receptor in airway infection
Institution: HOUSE EAR INSTITUTE LOS ANGELES, CA
Project Period: 2003/04/01-2007/03/31

DESCRIPTION (provided by applicant): Non-typeable *Haemophilus influenzae* (NTHi) causes infections in chronic obstructive pulmonary disease (COPD) and otitis media (OM). Both are characterized by inflammation. The molecular mechanisms underlying NTHi-induced inflammation remain poorly defined. Our long-term objective is to understand the molecular mechanisms by which the inflammatory response is induced and regulated in NTHi infections. Our recent studies showed that NTHi strongly activates nuclear factor-kappaB (NF-kappaB) via Toll-like Receptor 2 (TLR2). Because TLR2 expression in airway epithelial cells is low and overexpression of TLR2 greatly enhances NTHi-induced NF-kappaB activation, we hypothesize that NTHi up-regulates TLR2 via a specific signaling network. Our preliminary results indeed indicate that NTHi strongly up-regulates TLR2 via positive NF-kappaB and TGF-beta pathways and a negative EGFR-p38 MAPK pathway. Moreover, glucocorticoids synergistically enhance NTHi-induced TLR2 up-regulation. These encouraging results have thus laid a solid foundation for further investigation of the molecular mechanisms underlying NTHi-induced TLR2 upregulation (short-term objective). Aim 1. Determine the contribution of NF-kappaB and TGF-beta pathways to NTHi-induced TLR2 up-regulation by perturbing their signaling. Aim 2. Determine the contribution of EGFR-p38 MAPK pathway to NTHi-induced TLR2 up-regulation by perturbing their signaling. Aim 3. Determine the signaling mechanisms by which glucocorticoids synergistically enhance NTHi-induced TLR2 up-regulation by studying the effect of increased MKP-1 expression on NTHi-induced activation of p38 and TLR2 up-regulation. Significance: Understanding the signaling mechanisms underlying NTHi-induced TLR2 up-regulation will not only bring new insights into the regulation of inflammation, but will also open up novel therapeutic targets for modulating inflammatory responses in COPD and OM. Moreover, elucidating the molecular mechanisms by which glucocorticoids enhance NTHi-induced TLR2 up-regulation will provide instructive information regarding how to use glucocorticoids more appropriately in the clinic.

Grant: 1R01HL070860-01A1
Program Director: APPLEBAUM, DEBORAH
Principal Investigator: WEINTRAUB, NEAL L MD
Title: Endotoxin, Vascular Inflammation and Atherosclerosis
Institution: UNIVERSITY OF IOWA IOWA CITY, IA
Project Period: 2003/05/01-2007/04/30

DESCRIPTION (provided by applicant): Atherosclerosis, the most common cause of death in the United States, is a chronic inflammatory disorder. The sources of inflammation, and the mechanisms by which the inflammation leads to vascular disease, however, remain to be elucidated. Levels of circulating endotoxin, a glycolipid component of the outer membrane of Gram-negative bacteria, are markedly elevated during Gram-negative septicemia and lead to acute vascular inflammatory injury. Very recently, endotoxemia at much lower levels (i.e., >50 pg/ml) has been identified as a strong risk factor for atherosclerosis, particularly among smokers. Endotoxemia in apparently healthy subjects may result from chronic or recurrent infection, periodontitis, or breaching of epithelial barrier function. However, the extent of endothelial dysfunction caused by low levels of endotoxin, and its potential role in the pathogenesis of atherosclerosis, remain to be determined. Preliminary data from our laboratory indicate that relatively low levels of endotoxin (i.e., 1 ng/ml) increase the levels of reactive oxygen species (ROS), induce the pro-inflammatory cytokines interleukin-8 and monocyte chemoattractant peptide-1, and promote U-937 monocyte binding to human coronary artery endothelial cells. Similarly, very low levels of endotoxin (equal to approximately 30 pg/ml) induce inflammatory responses in human coronary artery smooth muscle cells and human blood vessel explants. These endotoxin-mediated pro-inflammatory effects are blocked by pre-treatment with HMG-CoA reductase inhibitors (statins) and epoxyeicosatrienoic acids (EETs), endothelium-derived metabolites of the polyunsaturated fatty acid arachidonic acid, which has potentially important implications for atherosclerosis and its treatment. Our hypothesis is that subclinical levels of endotoxin cause pro-inflammatory activation of human coronary artery endothelial and smooth muscle cells, and intact human blood vessels, and that these effects can be modulated by statins, EETs and fatty acids. Four specific aims are proposed, in which we will investigate the mechanisms of endotoxin signaling in vascular cells, the sources and consequences of endotoxin-induced ROS production, the regulation of endotoxin by specific binding proteins and enzymatic degradation, and the capacity of statins, EETs and other fatty acids to modulate endotoxin bioactivity. The proposed studies will provide novel insight into the mechanisms by which endotoxin-mediated vascular inflammation may contribute to atherosclerosis.

Grant: 1R01HL071522-01A1
Program Director: GAIL, DOROTHY
Principal Investigator: LEVINE, ANN M MD
Title: SP-A Receptor Regulation in Lung Innate Defense
Institution: CHILDREN'S HOSPITAL MED CTR CINCINNATI, OH
(CINCINNATI)
Project Period: 2003/07/01-2008/06/30

DESCRIPTION (provided by applicant): This application will test the hypothesis that surfactant protein-A (SP-A), a pulmonary collectin, plays a critical role in protecting the lung from bacterial infection by modulating surface receptors on alveolar macrophages. Preliminary data presented in this application provides a clear relationship between SP-A and complement receptor type 3 (CR3) providing a strong inference that SP-A effects are mediated through CR3. We propose that SP-A serves complex regulatory roles in the lung, binding to cell surface receptors present on alveolar macrophages influencing binding, uptake, and killing of microorganisms. CR3 is an important phagocyte receptor for recognition of microbial pathogens and is responsible for mediating phagocytosis, degranulation, and respiratory bursts by phagocytic cells. CR3 mediated phagocytosis is important in clearance of group B streptococcus (GBS) and Haemophilus influenza, both important pathogens in childhood disease. This application will utilize models in which the synthesis of SP-A is altered genetically, using SP-A^{-/-} and SP-A^{+/+} mice to determine if CR3 expression on alveolar macrophages is altered in the absence of SP-A. This application will test the central hypothesis that SP-A enhances phagocytosis and activates alveolar macrophages by modulating surface receptors mediating these events. Specific Aim 1 will test the hypothesis that SP-A regulates expression of CR3 on alveolar macrophages by mobilizing intracellular CR3 pools. Specific Aim 2 will test the hypothesis that SP-A binds to CR3 on alveolar macrophages and will determine the specific SP-A domain that enhances CR3 expression. Specific Aim 3 will test the hypothesis that SP-A opsonized GBS or H. influenza activate CR3 to enhance macrophage phagocytosis and oxygen radical production. Signaling pathways important in SP-A enhanced CR3 mediated phagocytosis will be studied in vitro using CR3 transfected cells and in vivo with alveolar macrophages from SP-A^{-/-}, CR3^{-/-}, SP-A^{-/-}CR3^{-/-} and wild type mice. These studies will help clarify the role of SP-A in innate defense of the lung and provide the basis for future therapies to maintain endogenous or supply exogenous SP-A to prevent morbidity from bacterial infection.

Grant: 1R01HL071544-01
Program Director: HASAN, AHMED A.K.
Principal Investigator: BOCK, PAUL E PHD BIOLOGICAL
CHEMISTRY
Title: Mechanism of Staphylocoagulase-activated Blood Clotting
Institution: VANDERBILT UNIVERSITY NASHVILLE, TN
Project Period: 2003/01/01-2007/12/31

DESCRIPTION (provided by applicant): The broad goal of the proposed studies is to define the molecular mechanism of the activation of human blood coagulation by the *S. aureus* protein, staphylocoagulase (SC), and the role of the mechanism in the pathogenesis of endocarditis. SC binds tightly to human prothrombin (Pro) and induces formation of a functional catalytic site in the zymogen without the usual strictly required peptide bond cleavages. This unique conformational activation mechanism is hypothesized to involve initial encounter of SC and Pro, followed by activation of the catalytic site and occupation of regulatory proexosite I in two or more discrete conformational changes. The mechanism may involve conformational linkage between proexosite I occupation and catalytic site activation, stabilization by high affinity binding of SC to the active conformation, and is unlikely to require insertion of the SC amino-terminus into a binding pocket in the Pro catalytic domain. The mechanism underlying the unique specificity of SC-Pro to convert fibrinogen (Fbg) to fibrin (Fbn) is hypothesized to bypass and inhibit the normal reactions of Pro activation. This mechanism is central to the propagation of platelet- Fbn-bacteria vegetations on heart valves in endocarditis. Fbg clotting activity of the SC-Pro/T complexes is hypothesized to involve specific recognition of Fbg as a substrate through expression of a Fbg-binding exosite on the SC-Pro/T complexes, in addition to changes in catalytic site specificity. Biochemical, biophysical, and structural approaches employing novel active site-labeled fluorescent derivatives of Pro are proposed to test hypotheses for conformational activation of Pro by SC and the basis of its specificity for conversion of Fbg to Fbn. Specific Aims are: (1) To determine the thermodynamic mechanism of conformational activation of Pro by SC; (2) To define the kinetic pathway of individual molecular events in conformational activation; (3) To elucidate the mechanism of specific recognition of Fbg as a substrate of SC-Pro/T complexes; and (4) To determine the three dimensional structures of SCI-327 bound to Pro/T species. The proposed studies are of fundamental significance in understanding how SC can circumvent the otherwise strict requirement for peptide bond cleavage in serine proteinase zymogen activation. The studies will provide new insight into the role of activation of Pro by SC in the pathogenesis of endocarditis and may ultimately allow therapy adjunctive to antibiotics to be developed based on inhibition of SC-activated blood coagulation.

Grant: 1R01HL072650-01
Program Director: BAROUCH, WINIFRED
Principal Investigator: YALLAMPALLI, CHANDRASEKHAR DVM
Title: Low birth weight, uterine infection, and nitric oxide
Institution: UNIVERSITY OF TEXAS MEDICAL BR GALVESTON, TX
GALVESTON
Project Period: 2002/12/30-2007/11/30

DESCRIPTION (provided by applicant): Urogenital infections and host factors are often associated with low birth weight, especially in minority populations. Most pathogens, including *Escherichia coli*, develop unique virulence mechanisms to colonize and invade the urogenital tract. Bacterial adhesins such as Dr fimbriae of *E coli* interact with host tissue receptors allowing ascending infection and associated complications. Nitric oxide (NO), a gaseous molecule with versatile functions including the modulation of infection and immunity, is reported to be produced by uteroplacental tissues. The goal of this project is to assess if NO modulates severity of uterine infection through the regulation of bacterial invasion into cells. We hypothesize that the NO system regulates the uteroplacental bacterial receptor, decay accelerating factor (DAF), and therefore bacterial invasion. We propose that this novel mechanism could play a role in severity of infection and perinatal morbidities such as low birth weight. These hypotheses will be tested by pursuing three specific aims. Specific Aim 1 will determine whether NO inhibits Dr+ *E coli* attachment and internalization into epithelial cells and whether this occurs through suppression of DAF expression. Sub-aim 1.1 will characterize NO production, NO synthase (NOS) enzymes in uterine epithelial cell lines, Ishikawa, RL-95 and HEC-1 cells. Sub-aim 1.2 will test the hypothesis if manipulation of NO synthesis in these cells will alter Dr +*E coli* attachment and internalization. Sub-aim 1.3 will test the hypothesis that the epithelial cell DAF protein and mRNA contents are regulated by NO system. Specific Aim 2 will establish that modulation of NO synthesis in rats will alter severity of infection through the changes in DAF content of the uterus and vasculature in experimental intrauterine infection. Sub-aim 2.1 will test the hypothesis that Dr* *E coli* or group B streptococcus (GBS) infection in uteroplacental tissues is reduced with increases in NO synthesis and is increased with the inhibition of NO synthesis. Sub-aim 2.2 will test the hypothesis that changes in DAF content of uteroplacental and vascular tissues are related to changes in NO synthesis. Specific Aim 3 will examine if inhibition of NO synthesis and experimental intrauterine Dr *E coli* or GBS infection results in fetal growth restriction in rats, and if so, whether NO donor can reverse the fetal growth restriction. Sub-aim 3.1 will test the hypothesis that inhibition of NO synthesis combined with intrauterine Dr+*E coli* or GBS infection has synergistic detrimental effects on fetal and placental growth. Sub aim 3.2 will test the hypothesis that NO donor can reverse the increases in DAF expression in uteroplacental and vascular tissues and in fetal growth restriction.

Grant: 1R01HL073525-01
Program Director: HARABIN, ANDREA L.
Principal Investigator: AYALA, ALFRED PHD REGULATORY BIOLO
Title: Regulatory Mechanisms of Acute Lung Injury
Institution: RHODE ISLAND HOSPITAL (PROVIDENCE, PROVIDENCE, RI
RI)
Project Period: 2003/09/01-2007/06/30

DESCRIPTION (provided by applicant): Of those trauma victims that survive the golden hours immediately following injury, upwards of 50% of these individuals develop some form of multiple organ failure (MODS). This occurs despite the provision of seemingly adequate fluid resuscitation, specific antibiotics, aggressive operative intervention, nutritional support and recently, antibodies to endotoxin as well as various anti-cytokine therapies. In this respect, acute lung injury is reported to be one of the most common forms of organ dysfunction in these individuals. Findings from a number of studies suggest "primed" neutrophils potentially play a key role in mediating this injury. Active suppression of neutrophil apoptotic death is thought to be a central aspect in the progression toward this pathological outcome. This implies that pro-apoptotic therapies might be efficacious. Alternatively, studies of lymphocytes in both septic and traumatized patients/animals indicate an increase in apoptosis in those cell populations and show that experimental anti-apoptotic therapies seem salutary. This raises important concerns about the design of pro- and/or anti-apoptotic therapies being considered for trauma patients. To address this controversy, we will test the hypothesis that the anti-apoptotic mechanisms shown to be beneficial in a clinically relevant model of sepsis, in which acute lung injury appears to be a minor pathological component, may prove to be deleterious in a model where acute lung injury is evident, such as hypotensive shock followed by septic challenge. To test this hypothesis, we have designed four Aims to examine the contribution of the neutrophils' apoptotic process to the development of acute lung injury by using selected knockout/transgenic mice as well as pharmacological interventions directed at genes or mediators thought to be involved in the regulation of A (sub o). The capacity of neutrophils, from mice of differing A (sub o) gene backgrounds and or inhibitor treatments, to produce acute lung injury will also be assessed by both PMN deletional and PMN add-back approaches, as will the role of macrophage/ lymphocytes in mediating the development of this state. It is our firm belief that the results of these studies will provide information that will not only allow us to better understand the pathobiology of acute lung injury but also its attenuation.

Grant: 1R01HL073967-01
Program Director: HARABIN, ANDREA L.
Principal Investigator: HUNNINGHAKE, GARY W MD BIOLOGY NEC:BIOLOG
NEC-UNSPEC
Title: Role of PI3K in Sepsis-induced Acute Lung Injury
Institution: UNIVERSITY OF IOWA IOWA CITY, IA
Project Period: 2003/09/01-2007/06/30

DESCRIPTION (provided by applicant): Human alveolar macrophages play a critical role in the response of the lung to sepsis and the development of acute lung injury. The major stimulus for the activation of these cells in the setting of sepsis is the recognition of microbial products such as endotoxin or lipopolysaccharide (LPS) from gram-negative bacteria and peptidoglycan (PG) from gram-positive bacteria. A number of signaling pathways play a role in the alveolar macrophage response to LPS. We have recently defined MAP kinase pathway roles in LPS signaling, transcription factor activation, and production of cytokines. We found that activation of all three MAP kinase pathways (ERK, JNK, p38) was needed for optimal release of TNF. Some of the results of these studies led us to investigate another important signaling pathway, the phosphatidylinositol 3-kinase (PI 3-kinase) pathway. Although this grant will not lose our interest in MAP kinase signaling, the overall focus of this project is to define the role of the PI 3-kinase pathway in LPS-mediated signaling in human alveolar macrophages. We wish to define the mechanisms for activation of PI 3-kinase (Aim 1), and the downstream effects of PI 3-kinase activity (Aim 2). The focus for Aim 1 (sphingolipids, reactive oxygen species (ROS) and the PI 3 phosphatase (phosphatase and tensin homologue deleted in chromosome ten, PTEN) derives from the information that ceramide is linked to PI 3-kinase activation (our studies), the described links between PI 3-kinase and ROS, and the fact that PTEN phosphatase activity reverses the effects of PI 3-kinase. The focus for Aim 2 (TNF and PGE2) derives from the information that LPS activation results in the coordinated release of first pro- and then anti-inflammatory mediators and we have already demonstrated a role for PI 3-kinase in this process. The clinical correlate of this sequence is the systemic inflammatory response syndrome (SIRS), which is often followed by a period of immunosuppression and, in some patients, acute lung injury. We have chosen to use TNF and PGE2 as molecular representations of SIRS (TNF) and the period of immunosuppression (PGE2). We wish to pursue this line of study because our preliminary data suggests that positive and negative regulation of the PI 3-kinase pathway is critical for the release of TNF and PGE2. Therefore, the overall hypothesis of this project is that PI 3-kinase activity is a molecular switch that regulates the transition from production of pro- (TNF) to anti- (PGE2) inflammatory mediators.

Grant: 1R01HL074175-01
Program Director: BANKS-SCHLEGEL, SUSAN P.
Principal Investigator: STANTON, BRUCE A
Title: P. aeruginosa pathogenicity and CFTR trafficking
Institution: DARTMOUTH COLLEGE HANOVER, NH
Project Period: 2003/08/15-2007/06/30

DESCRIPTION (provided by applicant): Our hypothesis is that P. aeruginosa decreases plasma membrane expression of CFTR in human airway epithelial cells by affecting the endocytic trafficking pathway. We hypothesize that a soluble, heat-sensitive factor secreted by P. aeruginosa decreases plasma membrane expression of wt- and deltaF508-CFTR by increasing endocytosis and/or decreasing endocytic recycling. Because identification of drugs that stimulate deltaF508-CFTR expression in the plasma membrane of airway epithelial cells is one of the major goals of CF drug discovery, it is important to know if P. aeruginosa has an effect on the plasma membrane expression of CFTR. Our preliminary studies demonstrate that P. aeruginosa causes internalization of plasma membrane wt-CFTR into endocytic vesicles in Calu-3 and MDCK cells. Furthermore, our data also indicate that P. aeruginosa reduces transepithelial CI secretion in polarized epithelial cells expressing wt-CFTR and deltaF508-CFTR. Thus, as long as CF patients are colonized with P. aeruginosa, drugs designed to increase the expression of deltaF508-CFTR in the plasma membrane may not be efficacious. Accordingly, the hypothesis to be tested is that P. aeruginosa decreases the apical plasma membrane expression of CFTR by affecting the endocytic trafficking of CFTR. To test this hypothesis we propose three specific aims: Specific Aim # 1. Test the hypothesis that P. aeruginosa decreases plasma membrane expression of wt-CFTR by affecting the endocytic trafficking pathway. Plasma membrane expression of CFTR depends in part on the relative rates of endocytosis and endocytic recycling. Accordingly, the goal of this specific aim is to determine if P. aeruginosa decreases plasma membrane expression of wt-CFTR by regulating the endocytic trafficking pathway; Specific Aim #2. Test the hypothesis that P. aeruginosa decreases plasma membrane expression of deltaF508-CFTR by affecting the endocytic trafficking pathway. The goal of this aim is to determine if P. aeruginosa reduces the efficacy of drugs that restore the trafficking of deltaF508-CFTR to the plasma membrane by regulating the endocytic trafficking pathway of deltaF508-CFTR; Specific Aim # 3. Test the hypothesis that a heat-sensitive factor secreted by P. aeruginosa affects the endocytic trafficking of CFTR. The goal of this specific aim is to identify the secreted factor. We will use a combination of complementary approaches, including molecular, biochemical, functional, and proteomic to elucidate how P. aeruginosa reduces the plasma membrane expression of CFTR. We anticipate that our studies may lead to the development of drugs that block the ability of P. aeruginosa to reduce plasma membrane expression of deltaF508-CFTR, and therefore will facilitate our long-term goal, which is to develop new therapies to increase the expression of deltaF508-CFTR in the plasma membrane.

Grant: 1R01HL075845-01
Program Director: COLOBINI-HATCH, SANDRA
Principal Investigator: FLYNN, JOANNE L
Title: Latent and Reactivation Tuberculosis
Institution: UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PA
PITTSBURGH
Project Period: 2003/09/19-2008/08/31

DESCRIPTION (provided by applicant): Tuberculosis is a major killer of Human Immunodeficiency Virus (HIV)+ persons worldwide. Compared to the 10% lifetime risk of a PPD+ person developing tuberculosis, an HIV+PPD+ person has a 10% annual risk of this disease. The interaction between HIV and Mycobacterium tuberculosis is not well-understood. By necessity, many of the studies to date have been performed in vitro or in a natural infection human setting, due to lack of an appropriate animal model. The non-human primate model can be used to address this interaction using Simian (human) Immunodeficiency Virus (SIV/SHIV) and M. tuberculosis co-infections. Specifically, in this application we will address the serious problem of latent tuberculosis and mechanisms by which reactivation of latent tuberculosis can occur. Many people infected with HIV are already latently infected with M. tuberculosis, and reactivation can occur at any level of immunocompromise. Using a cynomolgus macaque model of low-dose M. tuberculosis infection recently developed in our laboratory, we will explore latent and reactivation tuberculosis. Our non-human primate model appears to mimic human latent tuberculosis. We will examine and compare reactivation of latent tuberculosis in the macaque model using three different immunocompromising strategies. The reactivation triggers we have chosen are CD4 T cell depletion by antibody, SHIV co-infection, and TNF- α neutralization. By comparing the effects of each strategy on latent tuberculosis, in terms of clinical, immunologic and pathologic parameters, we can gain an understanding of latent tuberculosis, and this knowledge will be useful in devising strategies to prevent reactivation. We will also learn about the mechanisms by which HIV leads to reactivation, by comparing each of our three models. These studies are the first to study reactivation in an immunologically tractable animal model that is similar to human latent tuberculosis.

Grant: 1R01HL076955-01

Program Director: PEAUV, HANNAH H

Principal Investigator: MARTIN, WILLIAM J MD INTERNAL
MED:INTERNAL MEDICINE
OTHER

Title: Alveolar macrophage and mycobacteria

Institution: UNIVERSITY OF CINCINNATI CINCINNATI, OH

Project Period: 2003/09/22-2008/08/31

DESCRIPTION (provided by applicant): Profound immunosuppression as occurs in HIV-infected subjects is frequently associated with complicating mycobacterial infections. Mycobacterium tuberculosis is a worldwide pathogen and is a major cause of morbidity and mortality in both normal and immunosuppressed subjects. Non-tuberculous mycobacteria such as M. avium can be opportunistic infections that infect immunosuppressed individuals or subjects with underlying disorders such as pulmonary silicosis. Regardless of whether mycobacteria are acting as pathogens or opportunistic infectious organisms, mycobacteria in the lung first infect alveolar macrophages (AMs), the resident inflammatory cell of the alveolar spaces. AMs must be primed and activated by cytokines such as IFN-gamma or TNF-alpha to maximally respond to infectious organisms such as mycobacteria. Although it is recognized that AMs are central to the pathogenesis of mycobacterial disease, there are few studies that have examined the role of AMs in vivo in response to mycobacterial infection. We have developed a novel approach to "reconstitute" normal and activated AMs into the lungs of immunodeficient animals. We will use this new approach to test the hypothesis Deficiencies in the response of AMs to mycobacteria such as MAC or M. tuberculosis permit initial lung infection and subsequent dissemination during immunosuppression; conversely, correction of these AM deficiencies will restore alveolar immunity, control lung infection and prevent dissemination. We will examine the underlying mechanisms by which AMs respond in vivo to mycobacterial infection and will then use a variety of strategies to activate AMs for reconstitution to see if alveolar host defense is restored and infection eradicated. This will also test whether AMs activated by pro-inflammatory cytokines such as IFN-gamma mediate alveolar host defense to mycobacteria by AM-derived TNF-alpha. These Specific Aims include: 1) to determine the mechanisms by which normal AMs reconstituted into the lungs of immunodeficient mice restore alveolar host defense to mycobacteria and prevent dissemination, 2) to determine if proinflammatory cytokines such as IFN-gamma are essential for alveolar host defense to mycobacteria and to prevent dissemination, 3) to determine if ex vivo gene therapy to reconstituted macrophages results in persistent overexpression of pro-inflammatory cytokines such as IFN-gamma in vivo and improves alveolar host defenses to mycobacteria and prevents dissemination, and 4) to determine if the effects of pro-inflammatory cytokines such as IFN-? on alveolar host defenses to mycobacteria are mediated by AM-derived TNF-alpha. This proposal will test hypotheses in vivo not possible by other means and will determine whether reconstitution of normal or activated AMs is sufficient to restore alveolar host defense to mycobacterial disease despite the presence of ongoing systemic immunosuppression.

Includes Research Project Grants (RPGs)
Excludes Clinical Trials

Grant: 1R15HL066016-01A2
Program Director: PEARSON, GAIL DENISE
Principal Investigator: SHARMA, AVADHESH C PHD
Title: Endothelin Converting Enzyme in Sepsis: Cardiac Function
Institution: NORTH DAKOTA STATE UNIVERSITY FARGO, ND
Project Period: 2003/08/01-2006/07/31

DESCRIPTION (provided by applicant): The long-term objective of our group is to characterize an interactive role of vasoactive substances in sepsis-induced myocardial dysfunction and related cardiovascular pathologies. Chronic peritoneal sepsis in our rat model produces myocardial dysfunction in an isolated heart preparation and cardiomyocytes. Induction of sepsis also increases susceptibility of the isolated hearts to a calcium paradox-mediated myocardial injury. In vivo, we have demonstrated that induction of sepsis results in disproportionate alterations in the circulating levels of Endothelin-1 (ET-1) and nitric oxide byproducts (nitrite and nitrate, NOx). Recently we observed that inhibition of metalloprotease (endothelin-converting enzyme [ECE], which converts proET-1 to ET-1) at the time of induction of endotoxemia decreased the expression of myocardial inducible nitric oxide synthase (iNOS) and downregulated the expression of p38 mitogen-activated protein kinase (MAPK) twenty-four hours later. Therefore, our immediate objective in the present proposal is to test the hypothesis that sepsis-induced alteration in the biosynthesis of myocardial ET-1 (regulated by ECE-1) via MAPK-dependent or -independent mechanism(s) would affect NOS proteins and cardiac function. The following two specific aims are designed to address this hypothesis. Specific Aim 1: To determine if ECE-1 inhibition at the time of induction of sepsis would affect sepsis-induced myocardial dysfunction (decrease in the rates of left ventricular contraction and relaxation, i.e., + dP/dt and -dP/dt respectively) and the expression of p38MAPK and iNOS proteins at 12 and 24 h post sepsis (Year 1-2). Specific Aim 2: To determine if ECE-1 inhibition at the time of induction of sepsis via p38MAPK-dependent or -independent mechanism would affect myocardial function in an isolated heart preparation at 24 h post sepsis (Year 2-3). The specific aims were designed to assess if ECE-1 inhibition during sepsis would suppress sepsis-induced myocardial dysfunction characterized by downregulation of p38 MAPK and depressed expression of iNOS proteins. The novel aspect of the specific aims is that the results will provide evidence for a causal relationship between ET-1 biosynthesis and the expression of p38MAPK and iNOS proteins in the myocardium. An increased understanding of the underlying mechanisms during these two stages (12 and 24 h post sepsis) of sepsis will help design therapeutic interventions for early and late stages of sepsis.

Grant: 1R15HL071526-01
Program Director: CROXTON, THOMAS
Principal Investigator: CLEMANS, DANIEL L BS
Title: H. influenzae modulins in COPD airway inflammation
Institution: EASTERN MICHIGAN UNIVERSITY YPSILANTI, MI
Project Period: 2003/01/01-2005/12/31

DESCRIPTION (provided by applicant): Non-typeable *Haemophilus influenzae* (NTHi) is the most common bacterial cause of exacerbations of chronic obstructive pulmonary disease (COPD). These exacerbations are characterized by a brisk inflammatory response with the accumulation of polymorphonuclear leukocytes (PMN) in the lungs of patients with COPD. Essential to this inflammatory response is the expression and secretion of proinflammatory cytokines by host respiratory cells in response to NTHi. The mechanisms, by which NTHi stimulate a proinflammatory response by host respiratory cells and the progressive airway destruction in COPD, is unclear. Therefore, the overlying hypothesis of the proposed research is that secreted, nonlipooligosaccharide (LOS), NTHi proteins (i.e., modulins) stimulate the production of proinflammatory cytokines from human respiratory cells contributing to the endobronchial inflammation in COPD. The long-term goal of this project is to define the mechanisms by which specific secreted NTHi modulin(s) affect the recruitment and activation of effector cells in NTHi endobronchial infection. This goal will be met through identifying, purifying, and characterizing one such secreted modulin and its corresponding gene from model *H. influenzae* strain Rd and analyzing the modulin's role in respiratory inflammation in vitro. We will study the following Specific Aims to address the hypothesis. Specific Aim I will identify the secreted modulin from *H. influenzae* that stimulates a proinflammatory response in respiratory epithelial cells by 1) isolating the secreted modulin from *H. influenzae* strain Rd; 2) analyzing the secreted modulin gene from *H. influenzae* strain Rd; and 3) reconstituting the *H. influenzae* strain Rd modulin activity using the purified modulin. Specific Aim II will assess the distribution and expression of the secreted modulin gene from clinical isolates of NTHi. These studies will provide important insights into the mechanisms of the host inflammatory response in NTHi endobronchial infection of COPD and potentially identify novel therapeutic strategies to be employed in the treatment of this debilitating disease.

Grant: 1R15HL073835-01
Program Director: BANKS-SCHLEGEL, SUSAN P.
Principal Investigator: SAUER, KARIN PHD
Title: P. aeruginosa biofilm-specific proteins and regulators
Institution: STATE UNIVERSITY NEW YORK BINGHAMTON, NY
BINGHAMTON
Project Period: 2003/08/15-2006/07/31

DESCRIPTION (provided by applicant): Cystic fibrosis (CF) is one of the most common lethal genetic diseases among people of European descent, affecting 30,000 individuals in the United States. It is believed that chronic CF lung infections are caused by surface-associated, antimicrobial-resistant communities of microorganisms called biofilms with *Pseudomonas aeruginosa* being one of the principal pathogens. Current treatment strategies for CF infections, including frequent antibiotic treatment and chest physiotherapy, fail to clear these infections and biofilm bacteria persist in the lung despite intact host immune defenses. Recently, it has been suggested that therapeutic strategies directed towards biofilms may be successful in treating CF lung infections. Our research goal proposed herein is designed to elucidate the nature and identity of proteins that are unique to the biofilm mode of growth for the development of therapeutic strategies directed towards biofilms. Previous work in our laboratories has demonstrated that *P. aeruginosa* PAO1 undergoes a major shift in its cellular protein profile during biofilm development. This shift is most profound in biofilms grown for 3 and 6 days (maturation-I and maturation-II stage, respectively). We hypothesize that we will identify biofilm-specific proteins - important regulatory, virulence and resistance proteins - that are unique to the maturation-I and maturation-II biofilm stages. We expect that many of the biofilm-specific proteins are post-translational modified and have regulatory functions involved in signal transduction. Our goal will be accomplished by utilizing two-dimensional gel electrophoresis (2D/PAGE) combined with 2D-image analysis and protein identification. Biofilm-specific proteins will be identified by peptide mass fingerprinting using Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-ToF MS). Upon protein identification, functional proteomics will be used to provide an insight in signal transduction cascades: phosphorylated proteins will be immunoprecipitated and separated by 2D/PAGE. Comparative 2D-image analysis will reveal proteins that are uniquely phosphorylated in the protein patterns of biofilms grown to the maturation-I and -II biofilm stages. Uniquely phosphorylated, biofilm-specific proteins will then be analyzed by peptide mass fingerprinting and MALDI-ToF MS.

Grant: 1R21MH067070-01A1
Program Director: WINSKY, LOIS M.
Principal Investigator: BOEHM, GARY W BA
Title: Neurodevelopmental Effects of Perinatal Endotoxin
Institution: TEXAS CHRISTIAN UNIVERSITY FORT WORTH, TX
Project Period: 2003/06/23-2005/05/31

DESCRIPTION (provided by applicant): Research has shown that immune activation outside the brain may produce changes within the brain and alter subsequent behavior, potentially rendering one more susceptible to stress reactivity and mental illness. Among the many behavioral effects of peripheral immune activation is the potential for a negative impact upon learning and memory processes. A particularly important question that has yet to be thoroughly explored is to what extent do immune-mediated inflammatory events during early development lead to long-term changes in behavior? The specific aims of this exploratory/developmental grant are designed to develop a mouse model of perinatal immune activation and long-term consequences of immune activation upon anxiety, activity, and learning behavior. From these initial exploratory studies, a significant body of research may come, in which key developmental windows (i.e., critical periods) during which the brain is particularly sensitive to immune-induced insult, as well as the key proteins involved in such disturbances, may be identified. The specific aims include: 1). Investigating the long-term effects of perinatal endotoxin (derived from the bacterium *E. coli*) injections in two commonly used inbred mouse strains on well-validated tests of anxiety and locomotor activity, 2). Investigating the developmental effects of perinatal endotoxin in two commonly used mouse strains on well-established tests of spatial and non-spatial learning and memory, 3). Ascertaining that observed effects of perinatal endotoxin on performance in a given learning paradigm are, in fact, effects on learning, as opposed to effects on performance due to other factors (e.g., increased stress reactivity, increased anxiety, decreased motor activity, etc.), 4). Explore limited parameters of dose, developmental timepoint, and strain-related aspects of the behavioral effects of neonatal endotoxin exposure, and 5). Verify and explore the effects of peripheral endotoxin exposure on proinflammatory cytokine (factors induced by endotoxin that promote inflammation) gene expression within the neonatal brain. These studies will enable us to test the hypothesis that inflammatory cytokines induced in brain following immune activation in the periphery may alter the developmental trajectory of mammals exposed to unusual immune activation during important periods of neural development. Ultimately, this work may contribute to our understanding of important new perinatal risk factors.

Grant: 2P01NS035965-06A2
Program Director: MICHEL, MARY E
Principal Investigator: SIMON, ROGER P
Title: Molecular Mechanisms of Ischemia
Institution: EMANUEL HOSPITAL AND HEALTH CENTER PORTLAND, OR
Project Period: 1998/03/01-2008/04/30

DESCRIPTION (provided by applicant): The central theme of this program project is that potent endogenous mechanisms of neuroprotection are encoded in the genome and that the expression of a subset of these genes helps to determine whether cells survive ischemia. The scientific goals are to identify and characterize these genes and the neuroprotective pathways through which their protein products operate. The rationale for this approach is the understanding that the brain's response to injury is an active process that involves new protein synthesis. Identifying gene products that are endogenous neuroprotectants would contribute significantly to our understanding of the pathophysiology of ischemic neuronal injury and would point the way toward new therapeutic approaches to stroke and to related disorders, such as traumatic brain injury. For example, the discovery of a network of transcription factors and target genes that regulate ischemic tolerance in brain would advance pharmacologic efforts to mimic this effect. We will focus on in vivo and in vitro systems wherein endogenous neuroprotection has been induced and the brain has been made tolerant to subsequent ischemic injury (ischemic preconditioning and tolerance). The strategy for discovering neuroprotective genes in ischemia is to use mouse models of ischemic tolerance and microarray analysis to identify genes that are transcriptionally regulated in tolerance (Project 1). Identified genes will then be studied in vitro in models of ischemia and tolerance to characterize and confirm neuroprotective function (Project 2). Finally, gene products that are neuroprotective will be investigated by increasing or reducing their expression in mice in vivo, using pharmacologic and genetic approaches (Project 3). A Genomics Core (Core A) will provide Affymetrix microarray analysis to each project. Our collaborators at Pacific Northwest National Laboratory's Supercomputer and Bioinformatics Division will employ network analysis of gene clusters via conditional probability approaches and functional assignment of unknown genes using analysis of sequence similarities. The Administrative Core (Core B) will coordinate manuscripts, computer connections, data sharing, speaker travel, grants management, and statistical consultation for the interacting laboratories, as well as scientific consultation through internal and external advisory boards.

Grant: 1R01NS043355-01A1
Program Director: UTZ, URSULA
Principal Investigator: DALTON, DYANA K PHD
Title: Mechanisms of Regulation of Autoimmune Th1 CD4 T cells
Institution: TRUDEAU INSTITUTE, INC. SARANAC LAKE, NY
Project Period: 2002/12/15-2007/11/30

DESCRIPTION (provided by applicant): Autoimmune diseases are among the leading causes of death and disability in the United States and other developed countries. Autoimmune disease occurs when the immune system mistakenly unleashes its arsenal of defenses upon normal tissues. This response may be inadvertently triggered during an immune response to infection by microbes. The mechanisms for shutting down immune responses to microbial invaders are not understood. These regulatory mechanisms may play an important role in preventing autoimmune disease in healthy individuals. Thus, a greater understanding of mechanisms to downregulate immune responses may lead to better therapies for autoimmune diseases. Recently we have shown that the cytokine IFN-gamma and nitric oxide play important roles in turning off the immune response during infection with *Mycobacterium bovis* (BCG) and during experimental autoimmune encephalomyelitis (EAE), a mouse model for the autoimmune disease Multiple Sclerosis. Additionally, we and others have found that infection of mice with BCG prevents autoimmune diseases such as autoimmune diabetes of NOD mice and EAE. We have found that the protection of mice from autoimmune disease by mycobacterial infection requires the production of IFN-gamma and nitric oxide by the BCG-infected host. This raises several questions about whether the IFN-gamma-dependent mechanism for turning off Th1 CD4 T cell responses is part of the mechanism for protection of mice from autoimmune disease by BCG infection. We will address several issues in the current application. In Aim 1 we will examine the in vivo fate of Th1 EAE-inducing CD4 T cells after transferring them to uninfected and BCG-infected recipients. The effect of IFN-gamma and nitric oxide on the fate of these cells will also be determined by comparing wild-type, IFN-gamma KO, and nitric oxide-ablated donors and recipients. In Aim 2 we will elucidate the mechanism of IFN-gamma -dependent anergy and apoptosis of bystander CD4 T cells in vitro by BCG-infected splenocytes. Using this model these studies will determine (i) which spleen cells and molecules are required for suppression of CD4 T cells by IFN-?, (ii) whether BCG infected cells must present antigen to suppress CD4 T cells (iii) which soluble molecules mediate suppression (iv) whether Fas or CTLA-4 are involved in suppression and (v) whether the anergic CD4 T cells produce IL-2. This will allow us to relate this mechanism of CD4 T cell downregulation to known mechanisms. In Aim 3 we will determine the importance of live versus killed BCG, the route of infection, and the duration of protection against autoimmune disease by BCG infection. These studies will elucidate the mechanism of IFN-gamma -and nitric oxide dependent downregulation of Th1 CD4 cell responses.

Grant: 1R01NS045187-01
Program Director: NUNN, MICHAEL
Principal Investigator: RAMBUKKANA, ANURA PHD
Title: Targeting M. Leprae survival strategies in the PNS
Institution: ROCKEFELLER UNIVERSITY NEW YORK, NY
Project Period: 2002/12/15-2007/11/30

DESCRIPTION (provided by applicant): Leprosy remains an important global health problem, and represents a classical example of infectious neuro-degenerative diseases of the peripheral nervous system (PNS). Mycobacterium leprae infection of the Schwann cell, the glial cell of the PNS, is the primary cause for the nerve damage in leprosy. We have recently shown that the non-myelinating Schwann cell, but not the myelinated Schwann cell, preferentially harbors M. leprae, and thus serves as the intracellular niche for persistent infection. Because M. leprae is an obligate intra-cellular pathogen with the longest doubling time and a limited number of genes in its genome, the establishment of productive infection within non-myelinated Schwann cells is the key for bacterial survival. However, the mechanisms of M. leprae survival within Schwann cells are unknown. Targeting of M. leprae survival strategies will provide the rational to develop new therapeutics to combat the neurological injury and disease progression. To study these aspects, we used primary human Schwann cells (isolated and purified from human peripheral nerves) as a model, since they phenotypically resemble non-myelinated Schwann cells in vivo. Intracellular M. leprae in vitro maintain viability for several weeks without causing any apoptosis or cytopathic effect to Schwann cells. Microarray analysis using Affymetrix human GeneChips with cRNA prepared from primary human Schwann cells infected with viable M. leprae for 30 days, we showed that the majority of differentially expressed Schwann cells genes are (i) enzymes that regulate metabolic and respiratory functions, (ii) cell cycle regulators/inhibitors, (iii) growth/neurotropic factors, (iv) growth factor receptors and (v) associated transcriptional and signaling molecules. Therefore, we propose that once infected, M. leprae effectively use Schwann cell machinery on one hand to maintain the bacterial viability and the other hand to secure the intracellular niche for long-term bacterial survival by regulating Schwann cell growth. To study these, we will study the following: (1) M. leprae regulation of Schwann cell metabolic/catabolic functions, (2) Regulation of human Schwann cell cycle by M. leprae, and (3) M. leprae-induced growth/neurotropic factors and their effects on Schwann cell signaling, growth and functions. These studies should provide novel insight into the persistent M. leprae infection in the PNS, nerve damage in leprosy patients, and the basic biology of glial cells.

Grant: 1R01NS045316-01
Program Director: SHEEHY, PAUL A.
Principal Investigator: LING, ZAODUNG MD
Title: Prenatal Endotoxin as a Model of Parkinson's Disease
Institution: RUSH UNIVERSITY MEDICAL CENTER CHICAGO, IL
Project Period: 2003/01/01-2006/12/31

DESCRIPTION (provided by applicant): Current animal models of Parkinson's disease (PD) use high-dosages of dopamine (DA) neurotoxins to produce rapidly evolving lesions. We have recently shown that injection of the Gram (-) bacteriotoxin, lipopolysaccharide (LPS), into gravid female rats at embryonic (E) day 10.5 produces offspring born with fewer dopamine (DA) neurons. The 30-40% DA neuron loss routinely seen is still present after 4 months and presumed permanent. This DA neuron loss is similar to that seen in the PD patient since it is more pronounced in the lateral nigra and its ventral tier, spares the ventral tegmental area and calbindin immunoreactive DA neurons in the nigra, and is associated with reduced striatal DA and increased DA activity as well as tumor necrosis factor (TNFalpha). Exposure of these animals to the DA neurotoxin 6-hydroxydopamine (6OHDA) after 4 months, produces a greater inflammatory response and further DA cell loss. We hypothesize that the elevated DA activity and TNF seen in adult animals as a result of prenatal LPS treatment will lead to increased production of reactive oxygen species (ROS) that will eventually overwhelm ROS detoxification systems leading to further, progressive DA neuron loss as a result of aging or low dose exposure to 6OHDA. Based on this hypothesis we will study rats exposed to prenatal LPS and determine if further DA cell loss occurs with aging (through 21 months; Aim 1). Aim 2 will examine the combined effects of prenatal LPS and postnatal 6OHDA at various ages and determine if the combined effects of these two treatments is additive or synergistic and if the magnitude of the DA neuron loss as a result of these two neurotoxins increases with age. Whether or not animals exposed to prenatal LPS and 6OHDA at a young age exhibit a greater rate of DA neuron loss as they age compared with animals treated later in life, will be evaluated in Specific Aim 3. The successful implementation of these Specific Aims will address the notion that prenatal exposure to bacterial endotoxin is a risk factor for PD. Moreover, they would further demonstrate that prenatal infections such as bacterial vaginosis can interact with postnatal neurotoxin exposure to produce a gradual, protracted DA neuron loss that could be useful as a new animal model of PD.

Grant: 1R21RR018337-01
Program Director: FARBER, GREGORY K
Principal Investigator: KELLER, RICHARD A BA
Title: Single Cell DNA Fragment Sizing
Institution: UNIVERSITY OF CALIF-LOS ALAMOS NAT LOS ALAMOS, NM
LAB
Project Period: 2003/07/01-2006/05/31

DESCRIPTION (provided by applicant): We propose to develop a microscope-based imaging system for analysis of bacterial DNA fragments from single bacterial cells. Our approach eliminates the need for cell culturing common to other DNA fingerprinting methods, thereby reducing the analysis time from several days to hours. The proposed technique will allow DNA fragments, from a few hundred base pairs to millions of base pairs, originating from a single cell RFLP, to be sized. We envision many applications of this new capability in biomedicine to: more rapid diagnosis of infectious disease; determination of the source of an infectious disease outbreak; and measurement of the genotoxicity of drugs or environmental agents. In addition, this technique will impact biological research by providing a new measurement tool for single cell DNA analysis, as well as having immediate application to anti-bioterrorism, forensics, food safety, and agriculture. This technology will give researchers a powerful method for studying individual cells and organisms in the absence of averaging effects of ensemble measurements. Likewise, by making measurements on a number of single cells, information about the presence or extent of DNA heterogeneity will be established. The technique relies on performing all sample preparation reactions and analyses in an ultra-thin gel mounted on a microscope slide. Cell lysis, protein digestion, DNA restriction, and DNA staining, along with other reactions, will be carded out by diffusion of reagents into the gel. Staining conditions will be such that the fluorescence intensity is proportional to the fragment size. An electric field will be applied to the gel to electrophoretically separate the DNA fragments. Fluorescence from individual stained and separated fragments will then be detected and quantitated with a microscope-based, high sensitivity imaging system. The resulting DNA fragment size distribution histogram can be used as a fingerprint to identify individual organisms to the level of species and strain, detect damage in the DNA resulting from exposure to ionizing radiation or chemicals, or to monitor genetic variability. To demonstrate this technology we must complete the following specific aims: 1) assemble and characterize the apparatus and measurement approach; 2) develop and optimize the sample preparation chemistry; 3) demonstrate applicability to species and strain identification of representative bacteria.

Grant: 1R01TW006320-01
Program Director: MICHELS, KATHLEEN M
Principal Investigator: COREIL, JEANNINE PHD
Title: Stigma and Tuberculosis in Haitian Populations
Institution: UNIVERSITY OF SOUTH FLORIDA TAMPA, FL
Project Period: 2003/06/01-2006/05/31

DESCRIPTION (provided by applicant): Tuberculosis is a public health problem of global magnitude. In the U.S. its victims are primarily the poor, foreign immigrants and persons with AIDS. Efforts to control the disease are severely handicapped by the effects of social stigma, and further compounded by issues of race, social class, ethnic stereotypes, immigrant status and HIV co-infection. We will investigate the social dynamics of stigma in relation to TB in two populations particularly affected by all of these issues, Haitians in the U.S. and in Haiti. The study design will enable important comparisons across national settings that highlight the differential effects of political-economic context, as well as comparisons of stigma in a traditional public health clinic and an innovative culturally competent clinic. It will identify differences in stigma dynamics for active disease compared to latent infection, and will measure the impact of stigma on adherence to preventive therapy. Results of the study will enlarge our understanding of the role of social context on stigma enactment, and will help improve illness management strategies. An innovative study design applies the methodology of cultural epidemiology in an interactive quantitative-qualitative approach to developing locally valid measures of illness concepts and behavior. The approach combines the strengths of ethnography with traditional epidemiologic research. We will conduct an ethnography of TB stigma, a cross-cultural epidemiologic study, and a community trial of adherence to preventive therapy. Study sites are Broward County, Florida, and Leogane, Haiti. The methodology has been tested and refined in a broad range of geographic settings and illness problems, including tuberculosis. The research team brings ample qualifications to implement the proposed study, including preliminary studies of stigma and TB among Haitians in South Florida that led to the development of a culturally competent Haitian community clinic. The project has strong support from the Florida Bureau of Tuberculosis Control and Immigrant Health, the Broward County Health Department, Haitian Physicians Abroad and St. Croix Hospital in Haiti. Parallel cultural epidemiologic studies of illness-related stigma are being planned for several other countries. This will provide an opportunity to integrate the Haitian study with a larger, multi-country collaboration. However, this application is designed as a stand-alone project with independent research aims.

Grant: 2R03TW001235-04
Program Director: PRIMACK, ARON
Principal Investigator: CRAMER, WILLIAM A PHD
BIOPHYSICS:BIOPHYSICS-
UNSPEC
Title: Voltage-Gated Insertion of Colicin into Planar Bilayers
Institution: PURDUE UNIVERSITY WEST LAFAYETTE WEST LAFAYETTE, IN
Project Period: 2000/02/01-2005/11/30

DESCRIPTION (provided by applicant) Studies on membrane import and channel formation of the pore-forming colicins concern: the nature of (i) the large soluble --> membrane-bound structural transition undergone by colicins, toxins, and other membrane-active proteins, (ii) the surface-bound state that potentiates helix insertion, (iii) structure changes associated with voltage-gated channel formation, and (iv) the pathway of protein insertion into the membrane, and (v) the mechanism by which the MW = 65,000 colicins are translocated across the E. coli outer membrane. The structure of the colicin E1 channel domain, solved at atomic resolution, allows structure-based mutagenesis strategies to test models for structural transitions upon membrane-binding and channel formation. Single-Trp and -Cys mutants were used in fluorescence quenching and fluorescence resonance energy transfer to define the colicin channel bound in the membrane interfacial layer as an extended, flexible, two-dimensional helical net. Planar lipid bilayer experiments have been carried out in collaboration with the lab of Y. N. Antonenko (Moscow, Russia) to observe the kinetics of colicin channel formation and related properties. There have been 4 collaborative projects: (1) Colicin channel activity was photoinactivated in the presence of sensitizing dyes, and this effect depended on the presence of Trp495 in helix 9 of channel domain. Colicin photoinactivation will serve as an important model for study of photodamage of membrane proteins and photodynamic therapy, widely used in cancer treatment. (2) Colicin import and channel formation was found to be very sensitive to membrane anionic lipid content and to be "tuned" at a surface potential of -60 +/- 5 mV. (3) The channel activities of purified outer membrane receptors proteins were found to be occluded by exogenous colicin. (4) Preliminary experiments indicate that colicin E1 membrane-binding and channel formation is affected by the lipid interfacial dipole potential. It is proposed to simultaneously measure channel current and fluorescence with horizontal planar bilayers and to analyze the kinetics and pathway of voltage-gated colicin insertion into, and channel formation in, the membrane.

Grant: 1R03TW006122-01
Program Director: SINA, BARBARA J
Principal Investigator: NUDLER, EVGENY A MS
Title: Transcription Termination Control in Bacilli
Institution: NEW YORK UNIVERSITY SCHOOL OF NEW YORK, NY
MEDICINE
Project Period: 2002/12/15-2005/11/30

DESCRIPTION (provided by applicant) Decision between transcription elongation and intrinsic termination plays a crucial role in regulation of gene expression in bacteria. Classical examples of such regulation include attenuation mechanisms of amino acid biosynthetic operons in *Escherichia coli*. In those and most other cases the effector has been found to be a protein that either positively or negatively influenced intrinsic termination by directly affecting folding of the terminator stem-loop structure or affecting the formation of an alternate structure that competes with the stem-loop of the terminator. Recent work from this group describes a novel transcription attenuation mechanism, which controls riboflavin synthesis in *Bacillus subtilis*. This mechanism is unusual because small molecules, FMN and FAD, play a role of the effector that bind to the leader nascent transcript directly, changes its structure, and activate premature intrinsic termination. The long-term objective of the proposed work is to characterize in detail several biosynthetic operons in *B. subtilis*, which regulation resembles that of "riboflavin" pattern, i.e. the situation where sensing a small molecule by nascent RNA controls gene expression. Specific aims of this proposal are: 1. Complete studies on the transcription regulation of the riboflavin operon in *B. subtilis*. Genetic and biochemical experiments are proposed to address the role of a leader peptide in controlling the transcription of the riboflavin operon from *B. subtilis*. 2. Determine the mechanism of the transcription regulation of the thiamin operon in *B. subtilis*. Genetic and biochemical experiments are proposed to elucidate the structure and function of the leader regulatory region of the thiamin operon from *B. subtilis*. 3. Determine the mechanism of the transcription regulation of the S-box regulon in *B. subtilis*. Genetic and biochemical experiments are proposed to elucidate the structure and function of the leader regulatory region of the methionine operon from *B. subtilis*. This research will be done mainly in Moscow, Russia at the State Research Institute of Genetics and Selection of Industrial Microorganisms in collaboration with Dr. Alexander Mironov as an extension of the NIH grant # R01GM58750.

Grant: 1R03TW006234-01A1
Program Director: MCDERMOTT, JEANNE
Principal Investigator: FLANIGAN, TIMOTHY P MD
Title: Directly Observed Therapy(DOT) for TB and HIV in Kenya
Institution: MIRIAM HOSPITAL PROVIDENCE, RI
Project Period: 2003/09/01-2006/08/31

DESCRIPTION (provided by applicant): This research will be done primarily in Eldoret, Kenya at Moi University in collaboration with Lameck Diero, MD as an extension of NIH grant # RO1 DA13767. Tuberculosis is the leading cause of death in people living with HIV/AIDS worldwide, one of the earliest opportunistic infections occurring in conjunction with HIV, and is an accelerant for the replication of HIV and subsequent deterioration of the immune system. The intersection of the two epidemics in Sub-Saharan Africa has brought tremendous morbidity and mortality to countries already burdened by poverty and resultant poor health infrastructure. Debate has raged as to the feasibility of the introduction of HIV treatment in the form of ART in these countries. Yet, there already exists an infrastructure for delivery of such care in the form of the TB control systems. Directly observed therapy (DOT) is a crucial part of the recommended WHO strategy for TB control worldwide. DOT is a delivery system of care that ensures TB cure as well as the avoidance of drug resistance. The parallel between TB and HIV, in terms of requiring complex multidrug treatment regimens, suggests the concept of DOT as a delivery system that may be adapted successfully to use in HIV treatment. The parent grant of this FIRCA (Directly Observed HAART for Substance Abusers (DA 13767-01) is addressing use of the DOT care delivery system in HIV infected drug abusers in the United States. This FIRCA is to support a pilot feasibility project of combined DOT for the treatment of TB and HIV in Eldoret, Kenya. The advantages of this approach are multiple: 1.) TB is often the sentinel event that brings HIV infected patients to the health care systems of Kenya. 2.) Infrastructure in Kenya for TB DOT already exists. 3.) HIV seroprevalence in TB cases in Kenya is 60%. 4.) Despite the fact that patients may be cured of their TB, their mortality due to HIV is subsequently increased. Thus, the design of a DOT program that combines TB and HIV treatment not only serves a high-risk population at their point of entry into the health care system but also utilizes and expands an existing health care infrastructure approach (DOT). In addition, the longstanding collaboration between the Moi University Faculty of Health Sciences (MUFHS) and its US collaborating institutions supplies the ideal underpinnings for development of this project in Sub-Saharan Africa where its potential impact and generalizability is enormous.

Grant: 1R03TW006237-01
Program Director: MCDERMOTT, JEANNE
Principal Investigator: MCNEIL, MICHAEL R PHD
Title: D-arabinose synthesis in TB using Azorhizobium as a tool
Institution: COLORADO STATE UNIVERSITY-FORT FORT COLLINS, CO
COLLINS
Project Period: 2003/02/15-2006/01/31

DESCRIPTION (provided by applicant): Tuberculosis is a major opportunistic disease of AIDS which exacerbates the course of the illness. Furthermore, tuberculosis threatens AIDS caretakers and contacts and breeds drugs resistant strains of *M. tuberculosis*. Hence, new drugs, specific for *M. tuberculosis* are needed to help control the AIDS epidemic. D-arabinose formation is an ideal TB drug target as it is essential and specific for *M. tuberculosis* and D-arabinose is not found in humans. The pathway for formation of D-arabinose in *M. tuberculosis* has been determined by the PI; however, the genes have not been successfully identified. Thus, the purpose of this AIDS-FIRCA research is to identify the *M. tuberculosis* genes responsible for the synthesis of D-arabinose and determine the function of the encoded proteins. The breakthrough that has made both this research and the collaboration possible is the finding by the foreign collaborator, Dr. Holsters, of four genes in *Azorhizobium caulinodans* that synthesize D-arabinose (*A. caulinodans* is one of the very few other organisms in nature that synthesize D-arabinose besides mycobacteria). Furthermore, she discovered the existence of genes in *M. tuberculosis* homologous to three of the *A. caulinodans* genes. Therefore, we propose to identify the D-arabinose synthetic genes in *M. tuberculosis* by complementing specific gene knockout mutants of *A. caulinodans* with *M. tuberculosis* gene candidates. Further we propose to determine the function of each gene product by the identification of D-arabinose formation precursors in these specific gene knockout strains of *A. caulinodans* and by expression and enzymatic assay of the *M. tuberculosis* and *A. caulinodans* genes in *E. coli*. The genetics on *A. caulinodans* and the searches for D-arabinose precursors will be mostly performed at the foreign site while needed radioactive precursors and biochemical techniques and genetics in mycobacteria will come from the American site.

Grant: 1R03TW006264-01
Program Director: SINA, BARBARA J
Principal Investigator: LEE, JEAN C
Title: Biological relevance of capsule expression by *S. aureus*
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 2003/04/01-2006/03/31

DESCRIPTION (provided by applicant) *Staphylococcus aureus* is an opportunistic bacterial pathogen responsible for a diverse spectrum of human and animal diseases. Approximately 75% of *S. aureus* strains from humans are encapsulated. The prevalence of encapsulation among bovine isolates is variable, depending upon the geographic source of the isolate. An understanding of the role of the staphylococcal capsule in the pathogenesis of *S. aureus* infections is important. This FIRCA application is a supplement to Dr. Jean Lee's RO1 AI29040 grant to study the genetics of capsule production by *S. aureus*. The proposed research will be performed primarily in Argentina at the University of Buenos Aires by Dr. Daniel Sordelli and his colleagues. The objectives of the FIRCA project complement those of Dr. Lee's grant and are specifically related to specific aim #3. Under that aim, *S. aureus* isolates have been identified that lack the capsule genetic locus and carry an IS257-like element in its place. The proposed study will: 1) Investigate the loss of the cap5/8 gene cluster in NT *S. aureus* and the potential role of the IS257 element in such a mechanism. Whether copies of IS257 are associated with cap5/8 locus in *S. aureus* and whether loss of the cap5(8) genes can occur in vivo will be determined. The in vivo infections will include a systemic model of bacteremia leading to renal abscess formation and the mouse mastitis model of localized infection. 2) Determine whether CP5 or CP8 production enhances staphylococcal virulence in a mouse model of experimental mastitis. 3) Evaluate the effect of CP expression on *S. aureus* clumping factor A-mediated adherence to fibrinogen. The proposed research will permit further exploration of issues not covered by our currently funded projects, and the information that will emerge from it will expand our knowledge of *S. aureus* capsular polysaccharide biology.

Grant: 1R03TW006487-01
Program Director: MCDERMOTT, JEANNE
Principal Investigator: BRENNAN, PATRICK J
Title: M. tuberculosis Cell Wall Biogenesis; New Drugs; TB-HIV
Institution: COLORADO STATE UNIVERSITY-FORT FORT COLLINS, CO
COLLINS
Project Period: 2003/07/15-2006/06/30

DESCRIPTION (provided by applicant): The incidence of HIV-associated tuberculosis has been increasing worldwide since the beginning of the AIDS epidemic, and is expected to rise even further in the future, especially in developing countries. The accelerating and amplifying influence of HIV infection is contributing to the increasing incidence of disease caused by multidrug-resistant strains of *Mycobacterium tuberculosis*. Development of new drugs against tuberculosis is thus important for control of both of the infections. Mycobacterial cell wall is an attractive target for rational drug design against tuberculosis, due to the fact that it forms a protective, almost impermeable barrier, on the surface of mycobacteria. Some of the most effective drugs currently used for the treatment of TB affect components of its backbone - mycolylarabinogalactan-peptidoglycan (mAGP) complex. Our long-term goal is to identify processes and enzymes involved in the mAGP assembly. Possible AG biosynthetic gene cluster has been recently identified in the genome of *M. tuberculosis* and thus the specific aims of this grant proposal are: 1. Identify the genes involved in mycobacterial galactan biosynthesis within AG biosynthetic cluster and determine their biological functions via cloning, overexpression and subsequent biochemical characterization. 2. Establish the function of the putative ABC transporter within the AG biosynthetic cluster by way of preparation and phenotypic characterization of the mutants/conditional mutants. The approach will help define one of the more complex pathways in microbial biochemistry and reveal reactions that should be exploitable for drug development. The research will be primarily carried out at Comenius University, Faculty of Natural Sciences in Bratislava, Slovakia in collaboration with Katarina Mikusova, as an extension of the NIH grant A1-18357.

Grant: 1R21TW006347-01
Program Director: MICHELS, KATHLEEN M
Principal Investigator: VAN RIE, ANNELIES PHD
Title: Social Stigma of the New Tuberculosis
Institution: UNIVERSITY OF NORTH CAROLINA CHAPEL HILL, NC
HILL
Project Period: 2003/06/01-2006/05/31

DESCRIPTION (provided by applicant): For most of the 20th century, tuberculosis (TB) was in decline but since the mid 1980s, the trend has reversed and the number of TB cases globally now continues to increase. The "new" TB is characterized by HIV/-I-B co-infection and multiple drug resistant TB. In most parts of the world, TB and HIV epidemics are inextricably linked and call for an integrated approach of this dual epidemic. In developing countries, TB is the most frequent presenting opportunistic infection in people living with HIV/AIDS and TB is the leading cause of AIDS-related deaths. People fear TB, as it frequently heralds previously unsuspected HIV infection. The social stigma associated with the "new" TB is now compounded with the social stigma associated with HIV/AIDS. A better understanding of the changing social aspects of TB will be crucial for improved disease control. Clinical, biological and epidemiological interactions between TB and HIV have been studied extensively, but little is known about the psychosocial complexity of TB in HIV-infected individuals or about the social response of communities to TB in HIV/AIDS endemic areas. In this research project, a multidisciplinary team with clinical and operational research experience in the fields of behavioral science, epidemiology, sociology, ethnography, anthropology, infectious diseases and ethics propose to develop and validate a TB stigma scale for population-based assessment of TB stigma in the most southern part of Thailand, an area with a dual HIV/AIDS and TB epidemic. We will use the newly developed research instrument to obtain a better understanding of the key determinants of TB related stigma. We will measure the level of stigma experienced by TB patients in a cohort of active TB patients and correlate the stigma score to health-seeking behavior and adherence to TB therapy, two crucial elements of TB control. This project is the first attempt to integrate qualitative and quantitative research on stigma of TB in HIV endemic areas. The project will also initiate the collaboration between two universities (University of North Carolina, USA, and Prince of Songkla University, Thailand) to build research capacity on a subject of global importance.

Grant: 1R21TW006628-01
Program Director: ROSENTHAL, JOSHUA
Principal Investigator: OBERLIES, NICHOLAS H PHD
Title: Studies of the Flora and Predator Bacteria of Jordan
Institution: RESEARCH TRIANGLE INSTITUTE RESEARCH TRIANGLE PARK
NC
Project Period: 2003/09/30-2005/04/30

DESCRIPTION (provided by applicant): This R21 planning application proposes the development of an International Cooperative Biodiversity Group (ICBG) to investigate the biodiversity of the Hashemite Kingdom of Jordan (Jordan) for bioactive lead compounds useful for the treatment of human diseases and/or as viable agrochemicals (fungicides, larvacides, pesticides, etc.). The proposed studies build upon the historical successes, strong formal collaborations, and long-standing personal relationships between researchers at the Research Triangle Institute of North Carolina (RTI), the Virginia Polytechnic Institute and State University (VT), and the Jordan University of Science and Technology of Irbid, Jordan (JUST). Biodiversity proposed for investigation encompasses Jordanian plants and traditional botanicals, and the innovative area of Predator bacteria. Compound discovery focuses on three primary pharmacological areas: antimicrobial activity, anticancer activity, and central nervous system (CNS) activity. The six specific program areas are: 1) Flora for Lead Discovery from under-investigated Jordanian plants from diverse and unique environments (Botany and Biodiversity), 2) Flora for Herbal Drug Analysis based on safety and efficacy of medicinal plants used traditionally by the People of Jordan (Botany and Ethnobotany), 3) Predator Bacteria from the diverse topographical regions of Jordan (Microbiology and Biodiversity), 4) Bioassays for Lead Discovery in anticancer, antimicrobial, and CNS pharmacology and prescreening for general toxicity in the brine shrimp test (Pharmacology, Microbiology, and Toxicology), 5) Natural Products Chemistry to isolate, purify, and characterize bioactive lead compounds based on bioactivitybased fractionation and development of analytical reference compounds and standardization protocols for the analysis of commonly used Jordanian herbal medicinals (Pharmacognosy), and 6) Education, Training, and Economic Development for technology transfer in the form of course instruction and workshops in Jordan, training of Jordanian researchers and students in advanced chemical and pharmacological techniques at RTI and VT, and by stimulating and encouraging economic development opportunities resulting from the collective scientific output of the research team. Intellectual property rights and consideration of the People of Jordan have been agreed to in principle and plans for formalization of these agreements are outlined herein.