

FY00
NIH Extramural Support
in Bacteriology Research

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01AA012307-01A1
Program Director: LUCAS, DIANE
Principal Investigator: RAO, RADHAKRISHNA PHD
Title: MECHANISM OF ENDOTOXIN ABSORPTION IN ALCOHOLISM
Institution: MEDICAL UNIVERSITY OF SOUTH CAROLINA CHARLESTON, SC
Project Period: 2000/05/01-2000/08/31

DESCRIPTION: (Adapted from the Investigator's Abstract): The pathogenesis of alcoholic liver disease involves elevated plasma endotoxin and endotoxin mediated liver injury. Evidence from clinical and experimental studies suggests that elevated endotoxin level in alcoholism involves overgrowth of endotoxin producing bacteria and increased intestinal absorption of endotoxin. The investigators recently demonstrated that acetaldehyde the oxidative product of ethanol, known to be generated in the intestine, increases paracellular permeability in Caco-2 cell monolayers, an intestinal epithelial model. The acetaldehyde increase in permeability is mediated by a tyrosine kinase dependent mechanism and is associated with an inhibition of protein tyrosine phosphatase (PTPase) and increased protein tyrosine phosphorylation. The acetaldehyde effects on permeability was inhibited by L-glutamine, an amino acid considered for its therapeutic benefits in various gastrointestinal disorders. On the basis of preliminary results it is hypothesized that: 1) acetaldehyde dissociates protein complexes at the epithelial junctions by inducing tyrosine phosphorylation of b-catenin by regulating PTP1B (a PTPase), and 2) L-glutamine prevents acetaldehyde-induced increase in permeability by blocking the ability of acetaldehyde to inhibit PTP1B and increased tyrosine phosphorylation of b-catenin. Using the above mentioned model of intestinal epithelia, the investigators propose to determine: a) If acetaldehyde induces a dissociation of occludin/ZO-1 and E-Cadherin/b-catenin complexes. b) Whether acetaldehyde induces phosphorylation of b-catenin and specific tyrosine residues. c) Whether acetaldehyde inhibits PTP1B. d) If over expression of PTP1B delays acetaldehyde induced permeability and expression of phosphatase inactive PTP1B mutants decreases permeability, and e) If L-glutamine prevents acetaldehyde inhibition of PTP1B tyrosine phosphorylation of b-catenin and dissociation of E-cadherin/b-catenin complex. The information derived from these studies has the potential to expand our understanding of alcohol mediated increase in endotoxin absorption by identifying some of the mechanisms of acetaldehyde induced disruption of paracellular junctions and protection by L-glutamine.

Grant: 1R01AG015978-01A1
Program Director: PREMEN, ANDRE J.
Principal Investigator: WESTERINK, MA J MD
Title: ELDERLY IMMUNE RESPONSE TO PNEUMOCOCCAL POLYSACCHARIDE
Institution: MEDICAL COLLEGE OF OHIO AT TOLEDO TOLEDO, OH
Project Period: 2000/04/01-2005/03/31

Infection is one of the leading causes of morbidity and mortality in the elderly. *Streptococcus pneumoniae* is the organism most commonly isolated from elderly patients with pneumonia. Increased susceptibility to infections that occur in the elderly has been attributed to deteriorating health, decreased pulmonary function and a functional decline of the immune system. The immune system is unique in that it may be manipulated to achieve a desirable response. Studies in aged mice demonstrate both quantitative and qualitative changes in the immune response to T-independent type 2 (TI-2) antigens. Reports indicate age related loss of affinity, fine specificity and protective immunity are associated with a molecular shift in V gene usage and changes in cytokine profile. Studies of the in vivo immune response in elderly have been limited to vaccine efficacy studies and quantitative analysis of the magnitude and duration of the post-vaccination antibody response. The results of these studies suggest that despite adequate quantitative immune response the elderly show decreased vaccine efficacy. Current knowledge concerning the aging immune response to TI-2 antigens is mostly based on animal models and may not be applicable to humans. Human studies are fragmented and address quantitative and qualitative immune response as separate issues. We propose to study and characterize the immune response to *S. pneumoniae* capsular polysaccharide in the elderly. We will focus on both quantitative and qualitative changes in the immune response on molecular and functional levels. The quantitative immune response, isotype and IgG subclass, will be correlated with opsonophagocytic activity. We hypothesize that the discrepancy between the quantitative and qualitative immune response in the elderly results from altered V region sequence. We will characterize the immunoglobulin gene usage pattern and V-D-J joint diversity of the antibody response to pneumococcal polysaccharides (PPS) of serotypes 4 and 14 in elderly and young adults. This will be accomplished by gene family specific ELISA and by isolating single responding cells and determining the sequence of the V chains. Second, we propose to evaluate the influence of soluble regulatory factors on the aging immune response. The reconstituted SCID mouse model will be used to study the aging human B cell response to PPS 4 and 14 in a controlled cytokine environment allowing us to differentiate altered response intrinsic to the B cells versus altered responses secondary to environmental factors such as cytokines. The results of these studies will form the essential baseline for the rational development of vaccine and adjuvant strategies for the elderly.

Grant: 2P01AI036359-04A1

Program Director: ASH-SHAHEED, BELINDA

Principal Investigator: LAMM, MICHAEL E MD
CHEMISTRY:CHEMISTRY-
UNSPEC

Title: MUCOSAL IMMUNITY AND INFECTION

Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH

Project Period: 1995/09/30-2005/08/31

OVERALL DESCRIPTION (Adapted from application): This Program Project Application is submitted by an interactive group of basic and clinical scientists who propose to continue to study the mucosal immune system in relation to parasitic, bacterial and viral infections. The proposed research focuses on the broad areas of pathogenesis, prevention and therapy of important diseases caused by different classes of infectious agents that infect the gastrointestinal, genital and respiratory mucosae. Project 1 addresses *Entamoeba histolytica* and amoebiasis, a leading cause of parasitic death and morbidity worldwide. The specific studies focus on the galactose-inhabitable lectin, that mediates the binding of *Entamoeba* to the intestinal epithelium. The goal is to identify immunogenic subdomains of the lectin that can be used to develop an effective oral subunit vaccine. The second project is on *Helicobacter pylori*, the major cause of peptic ulcer disease. Based upon studies of pathogenesis and mechanisms of immune defense, a major goal is to develop prophylactic and therapeutic vaccines that do not elicit an untoward inflammatory immune response, a major goal is to develop prophylactic and therapeutic vaccines that do not elicit an untoward inflammatory immune response. Project 3 investigates how mucosal IgA antibodies can counter HIV at epithelial surfaces that are the portals of entry for sexual transmission of this virus. Monoclonal IgA antibodies to HIV, both extracellular and intracellularly. The mechanisms of action of such protection will be studied. The results may further the design of an effective mucosal vaccine for this sexually transmitted disease. The fourth project investigates IgA nephropathy, the most common type of glomerulonephritis, that is associated clinically with respiratory infection. The roles that normal and aberrant IgA glycosylation, virus-specific T cells and glomerular mesangial cells play in disease pathogenesis will be investigated in a post infection mouse model in nephritis-sensitive and nephritis-resistant strains. The four projects are supported by administrative and hybridoma cores. The insights to be gained from this PROGRAM Project may be broadly applicable since many infections involve mucous membranes, either as sites of infection or as portals of entry into the host.

Grant: 1P01AI046518-01
Program Director: SAVARESE, BARBARA M.
Principal Investigator: ANDERSON, DEBORAH J
Title: IMMUNITY TO STDS IN THE HUMAN MALE GENITAL TRACT
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 2000/09/15-2004/08/31

Sexually transmitted diseases (STDs) cause extensive morbidity and are epidemic in many developing countries and in certain segments of the US population. Little is known about immune defense mechanisms of the male urogenital tract that normally limit STD infections or that can be induced to protect against transmission of STD pathogens. Such information would facilitate the development of vaccines and other strategies to prevent STDs. This Program Project application addresses several aspects of this important research area. Three research projects and two service cores (Administrative and Clinical) are proposed. Project 1 (Dr. Anderson, PI) will investigate humoral and cellular acquired immune responses in the male genital tract and their regulation. A special focus of this project will be the molecular definition and functional studies of immunoregulatory molecules and changes in their expression during infection. It is hypothesized that the male urogenital tract is an inductive site for local humoral immunity, but that cellular immune responses are tightly regulated. Project 2 (Dr. Quayle, PI) addresses the role of epithelial defensins (HD-5, HBD-1 and HBD- 2) in early host-pathogen interactions in the male urogenital tract. This project will characterize expression patterns and secreted forms of defensins in normal men and men with STDs, their activity against STD pathogens, and the role of defensins in leukocyte recruitment to the mucosa. Project 3 (Dr. Toribara, PI) will investigate mucin expression at various sites in the male genital tract, and address the hypothesis that mucins play an important role in mucosal immune defense. Investigators working on Projects 1 (acquired immunity) and 2 (defensins) will collaborate with investigators working on Project 3 (mucins) to define functional interactions between classic immunological mediators (cytokines, immunoglobulins, lymphocytes, defensins) and mucins present in the male genital tract. The Administrative Core will provide infrastructure support for the program. The Clinical Core, codirected by Drs. J. Pudney and P. Rice (PI of the Boston STD-CRC), will provide five services: 1) a male genital tract tissue bank for studies on cellular distribution and expression of defense molecules in different regions of the male genital tract; 2) immortalized epithelial cell lines from prostate, urethra and seminal vesicles and STD organisms for in vitro studies of effects of infection on gene regulation of defense and immunoregulatory molecules; 3) urethral and prostatic secretions from men with specific STDs and controls for studies on regulation of defense mechanisms by natural infections in vivo; 4) a PCR service for screening tissues and clinical samples for specific STD pathogens; and 5) database and statistical support.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2R01AI012601-24
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: LOVETT, MICHAEL A MD INTERNAL
MED:INFECTIOUS DISEASE
Title: IMMUNE MECHANISMS IN EXPERIMENTAL SYPHILIS
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 1978/06/01-2005/01/31

To study the role of the rare *T. pallidum* (Tp) outer membrane (OM) spanning proteins in pathogenesis and immunity, we isolated Tp OM vesicles (OMV). OMV porin activity resides in a 31 kDa protein, Tromp1. Native Tromp1 is 31, 510 Da, and is processed from a 33,571 Da precursor. While native Tromp1 is hydrophobic, and trimeric, urea creates a hydrophilic monomer, indicating that the hydrophobicity of the native protein is conformationally determined. Monomeric rTromp1 has no poring activity and is hydrophilic. Renatured rTromp1 is trimeric and hydrophobic, with porin activity like native Tromp1. Renatured rTromp1 formed intra membranous particles in proteoliposomes. OMV were used to generate mouse antiserum that showed a 100% killing endpoint titer 32 times greater than time of immune rabbit serum (IRS). The OMV ant- serum bound Tromp1, Tromp2, and four lipoproteins were fully removed, while Tromp antibodies remained. The adsorbed serum showed no reduction in its high-titered treponemicidal activity, suggesting that Tromp1 and/or Tromp2 are the targets of this activity. Treponemicidal activity greater than that found in IRS has never previously been demonstrated. Our specific aims are therefore: 1. Determine the significance and basis of OMV induced treponemicidal antibodies. We will learn if OMV can convey protection in experimental syphilis. The possibility that treponemicidal antibodies. We will learn if OMV can convey protection in experimental syphilis. The possibility that there are relevant OMV proteins other than Tromp1 and 2 will be rigorously considered. The role of OMV in pathogenesis will be addressed by use of adherence and invasion assays. 2. Determine the role of Tromp1 in pathogenesis and immunity. Mass spectrometry adapted to nanogram amounts will be used to insure that rTromp1 faithfully duplicates the primary structure of native Tromp1. The ability of renatured rTromp1 and native Tromp1 to induce treponemicidal antibodies and protective immunity will be assessed, along with the role of Tromp1 in pathogenesis. 3. Determine the role of Tromp2 in pathogenesis and immunity. Tromp2 has conformationally determined hydrophobicity, but lacks porin activity. Tromp2 is considerably less abundant than Tromp1. Definitive determination of the primary structure of mature Tromp2 will be used as the basis for creating a rTromp2 whose primary structure is identical to the native protein. rTromp2 will be renatured and studied as outlined for rTromp1.

Grant: 2R01AI013446-21
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: WYRICK, PRISCILLA B PHD ANATOMY:ANATOMY
UNSPEC
Title: VIRULENCE FACTORS OF CHLAMYDIAE
Institution: EAST TENNESSEE STATE UNIVERSITY JOHNSON CITY, TN
Project Period: 1995/04/01-2005/03/31

Chlamydia trachomatis serovars D-K are the most common cause, in the USA and worldwide, of bacterially-acquired sexually transmitted diseases and their sequelae, including prostatitis, epididymitis, pelvic inflammatory disease, ectopic pregnancy and sterility. Chlamydial diseases are insidious and they constitute significant primary, secondary and tertiary health concerns in which women bear a special burden because of their increased risk of adverse reproductive consequences. The goal of this laboratory for 25 years has been to try to understand the basic biology of chlamydial growth in its host epithelial cell in order to understand the infectious process and to permit dissection of the cellular and molecular consequences of persistent infection, since the majority of chlamydial tubal disease appears to result from chronic subclinical, persistent infection. This proposal is a continuation of on-going efforts to understand the crucial attachment/entry steps, the signals in chlamydiae-infected epithelial cells which trigger neutrophil chemotaxis--since a prolonged inflammatory response to persistent chlamydial antigens is believed to be responsible for the damage and sequelae, and hormone modulation of entry and signaling of neutrophils. In Aim 1, the chlamydial envelope-associated hsp70 and its co- chaperonins GrpE and DnaJ will be incorporated into liposomes, along with known and suspected adhesins, to define the role of hsp70 in entry and, in Aim 2, help identify the receptor which functions with newly identified, estrogen-responsive receptor accessory proteins. Also in Aim 2, the swine *C. trachomatis* S45 isolate- swine genital tissue model of infection will be developed to dissect hormone modulation of entry and neutrophil signaling (Aim 3). In Aim 3, a comparison will be made of chlamydial and chemokine signals triggering neutrophil chemotaxis to polarized HeLa cells normally and persistently infected with non-invasive, asymptomatic serovar E versus invasive, symptomatic serovar L2. Finally, in Aim 4, cryo-electron microscopy and density gradients will be used to show that chlamydial antigen secretion and trafficking can occur via vesicles pinched off from the chlamydial inclusion.

Grant: 2R01AI014107-25
Program Director: KLEIN, DAVID L
Principal Investigator: HOLMES, RANDALL K MD
MICROBIOLOGY:MICROBIO
OGY-UNSPEC
Title: CONTROL OF BACTERIAL TOXINS BY VIRUSES AND PLASMIDS
Institution: UNIVERSITY OF COLORADO DENVER/HSC AURORA, CO
AURORA
Project Period: 1976/09/30-2005/03/31

DESCRIPTION (Adapted from the Applicant's Abstract): The long term goals of this project are to determine molecular mechanisms for virulence regulation in pathogenic bacteria and to develop new methods to treat bacterial infections. The investigators will study *Corynebacterium diphtheriae*, a paradigm for toxin-mediated bacterial infections, and *Mycobacterium tuberculosis*, a prototype for intracellular bacterial infections. These very different bacterial pathogens produce closely related, iron-activated, global regulatory proteins that govern virulence: the diphtheria toxin repressor (DtxR) and the iron-dependent regulator (IdeR), respectively. The investigators will determine the molecular basis for function of DtxR, IdeR and the homologous regulator SirR from *Staphylococcus epidermidis*. The investigators will use structure-based design to develop new antimicrobial drugs called "super-activators" that will target DtxR, IdeR or their homologs; activate them by iron-independent mechanisms; and inhibit production of virulence factors that are negatively regulated by iron- and DtxR-related repressors. The development of IdeR as a novel target for antimicrobial therapy could address the urgent global need for improved treatment of tuberculosis. The investigators will characterize the genes and gene products that are iron-regulated and under control of DtxR and IdeR, both to provide new insights into the pathogenesis of diphtheria and tuberculosis and for development of additional classes of antimicrobial agents. Specific Aim 1 will analyze structure and function of DtxR, IdeR and SirR. The investigators will investigate the molecular basis for repressor-operator interactions, for iron-independent super-repressor activity, and for domain function in biological activity of these regulatory proteins. Specific Aim 2 will characterize the DtxR and IdeR regulons in *C. diphtheriae* and *M. tuberculosis*. The investigators will develop an allelic exchange system for *C. diphtheriae*, characterize the DtxR and IdeR regulons by proteomic and molecular genetic methods, assess physiological functions of DtxR and IdeR domain 3, and investigate atypical phenotypes among clinical isolates of *C. diphtheriae*. Specific Aim 3 will develop super-activators of DtxR and IdeR by structure-based design. The investigators will design combinatorial peptide libraries, test them for super-activator function, identify individual peptides with activity, determine the structural basis for that activity, and develop better super-activators by iterative application of these methods. The investigators will also use molecular genetic methods to identify novel mechanisms for super-repressor activity and new lead compounds for development as tools against these bacterial infections.

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Excludes clinical trials

Grant: 2R01AI015035-23
Program Director: BAKER, PHILLIP J.
Principal Investigator: WINKLER, HERBERT H PHD
Title: PERMEABILITY OF THE EPIDEMIC TYPHUS RICKETTSIA
Institution: UNIVERSITY OF SOUTH ALABAMA MOBILE, AL
Project Period: 1979/01/01-2004/12/31

This proposal is focused on the characterization of transport systems in *R. prowazekii*, the etiologic agent of epidemic typhus. *R. prowazekii* has only 834 genes. Such a small genome size is possible because many intermediates required for their metabolism need not be synthesized, but are transported from the cytoplasm of the host cell by unusual transport systems that often have no counterparts in free-living bacteria. Aim I. Structure and function of the ATP/ADP transport system. Using cysteine scanning mutagenesis and the derivatization and cross-linking of cysteine residues, we will characterize essential residues and the packing of transmembrane helices that configure the formation of aqueous channels and active sites. Our biodiversity approach will be extended and a new effort to select for the TLC system in *E. coli* will be initiated. Aim II. Characterization of transport systems with multiple homologous genes. II.A. Functions of the four *tlc* genes paralogous to that for ATP/ADP transport. II.B. Function of the seven paralogous proline transport genes. We will determine the nature of the transport systems encoded by these twelve paralogous rickettsial genes when cloned into *E. coli* and in native *R. prowazekii*. We will establish their natural substrates in rickettsiae, their energy coupling, and by using knock-out recombinants determine whether all of these twelve paralogs are essential. Aim III. ABC-transport systems in rickettsiae: focused on glutamine transport. Two hallmarks of the ABC-transport system are remarkable high affinity and the ability to establish a very high thermodynamic transmembrane gradient of substrate. Such a transport system seems inappropriate in *R. prowazekii* living in cytoplasm. We will focus on the glutamine transport system. The alternative hypotheses that we will investigate are: 1) the rickettsial glutamine-ABC is high affinity, always works at saturation levels of host cytoplasmic glutamine, and does not care about inefficiency because there is plenty of ATP around; 2) high affinity of this transport system is appropriate because, although the total concentration of glutamine in host cytoplasm is high, the free concentration available to the rickettsia is low because of competition with tRNA; 3) these genes in *R. prowazekii* are not used to transport glutamine but are in the process of mutational melt-down; and 4) the rickettsial glutamine transport system is now non-functional, with substrate recognition residing totally in the membrane protein.

Grant: 2R01AI015136-22

Program Director: MILLER, MARISSA A.

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

Title: MOLECULAR PATHOLOGY OF LPS-INDUCED INJURY

Institution: SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA

Project Period: 1978/08/01-2005/04/30

DESCRIPTION (Adapted from the Applicant's Abstract): The proposed studies are designed to characterize mechanism(s) of septic shock, which still remains a major cause of morbidity and mortality in the United States. The dysregulated response of innate immunity caused by bacterial infections is responsible for the clinical manifestation of septic shock syndrome. This immune response leads to upregulation of host defense but, unfortunately, the same response also causes cellular injury and septic shock. The PI and his colleague plan to use immunologic, molecular biological and biochemical approaches to investigate mechanisms associated with activation of the innate immunity by bacterial products. LPS will be used as a prototype of bacterial toxins in order to characterize the functional role of the components of LPS receptor complex (LPSRC), e.g. CD14 and Toll-like receptors (TLRs), in activation of myeloid lineage cells. To address this question, quantitative LPS binding assays and other biochemical techniques will be used to define the sequence of events occurring at the cell surface after LPS binds to CD14. Specifically, they will attempt to identify which, if any, candidate protein within the complex (TLRs, MD-2 etc.) binds LPS and whether LPS binding causes composition changes in the LPSRC. Intracellular signaling events induced by ligation of LPSRC will be characterized with a specific focus on upstream components that link LPSRC to the p38 and JNK kinase pathways. The results of in vitro studies will be used to guide the proposed in vivo studies to investigate the functional role of LPSRC in a rabbit model of endotoxic shock that closely resembles septic shock in humans. To accomplish the proposed in vivo studies, the PI and his colleagues suggest the generation of a panel of monoclonal antibodies against different components of rabbit LPSRC. The results of these studies, if successfully accomplished, may lead to the development of new strategies to control the innate immune responses in bacterial infection.

Grant: 2R01AI015495-18
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: MC MURRAY, DAVID N PHD
MICROBIOLOGY:MICROBIO
OGY-UNSPEC
Title: DIETARY DEFICIENCIES AND TUBERCULOSIS VACCINE EFFICIENCY
Institution: TEXAS A&M UNIVERSITY HEALTH SCIENCE COLLEGE STATION, TX
CTR
Project Period: 1982/09/30-2005/01/31

The ultimate aim of this project is to elucidate the mechanisms by which protein deficiency interferes with vaccine-induced resistance to pulmonary tuberculosis. New tuberculosis vaccines will be tested, ultimately, in malnourished humans. Thus, the focus of this project has never been a more urgent research priority. Previous work with a highly relevant guinea pig model, employing low-dose aerosol exposure to virulent *Mycobacterium tuberculosis*, has revealed diet-induced defects in trafficking, activation, and interactions of immune cells. The recent acquisition of cDNA clones for guinea pig chemokines (MCP-1, IL- 8, RANTES) and cytokines (IFN γ , TNF α , TGF β , and IL- 1 β) provides a unique opportunity to apply molecular biological approaches to test four hypotheses: (a) protein deficiency affects trafficking of immune cells into inflammatory exudates by interfering with the production/function of chemokines; (b) abnormal granuloma formation in protein-deficient guinea pigs results from alterations in the production/function of TNF α ; (c) failure of immune lymphocytes from protein- deficient guinea pigs to activate macrophages to suppress intracellular *M. tuberculosis* is due to decreased production/function of paracrine (IFN γ) or autocrine (IL- 1 β , TNF α) cytokine signals; and (d) TGF β -mediated suppression of T lymphocytes and/or deactivation of macrophages leads to loss of control of intracellular mycobacteria in protein deficiency. Recombinant proteins and polyclonal antibodies will be produced for each of the chemokines and cytokines. Protein- deficient and well-nourished, BCG-vaccinated or nonvaccinated guinea pigs will be challenged by the pulmonary route and levels of these molecules assessed in freshly isolated or cultured cells by Northern blot and RT-PCR (for mRNA), or by bioassay (TNF α , TGF β) or ELISA. A tuberculous pleuritis model, previously established in the laboratory, and bronchoalveolar lavage will be used to assess the role of chemokines by instillation of recombinant chemokines or specific anti-chemokine antibodies. The effects of recombinant TNF α or anti-TNF α on granuloma formation will be determined. TGF β activity will be blocked in vivo by the injection of anti-TGF β antibodies or recombinant decorin. These experiments will provide important new insights into the contributions of these molecules to loss of tuberculosis vaccine efficacy observed in malnourished subjects.

Grant: 2R01AI016478-21
Program Director: MILLER, MARISSA A.
Principal Investigator: BERG, HOWARD C PHD CHEMISTRY:PHYSICA
Title: SENSORY TRANSDUCTION IN BACTERIAL CHEMOTAXIS
Institution: HARVARD UNIVERSITY CAMBRIDGE, MA
Project Period: 1986/12/01-2004/11/30

Abstract Text Not Available

Grant: 2R01AI016692-20
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: NORGDARD, MICHAEL V
Title: MEMBRANE PROTEINS OF TREPONEMA PALLIDUM
Institution: UNIVERSITY OF TEXAS SW MED DALLAS, TX
CTR/DALLAS
Project Period: 1980/05/01-2005/04/30

Syphilis is a chronic, complex sexually transmitted disease caused by the spirochetal bacterium *Treponema pallidum*. That syphilis remains an alarming global public health problem and is a cofactor for the transmission of HIV underscore the importance of continued studies to elucidate its complex pathogenesis. Such studies are requisite for the development of a syphilis vaccine, an important element of the syphilis eradication initiative. Given that the membrane system of *T. pallidum* serves as both the physical and functional interface with the host, studies to elucidate the structure, function, and immunology of *T. pallidum* membrane proteins continue to be essential for the future design of novel syphilis intervention strategies. To this end, the Specific Aims of this proposal are: (1) To refine and implement a new chemotaxis assay for *T. pallidum*, with emphasis on elucidating potential chemoattractants that may facilitate tissue dissemination by *T. pallidum*; (2) To assess whether the Mg1B lipoprotein of *T. pallidum* is a receptor for glucose, the principal carbon and energy source for *T. pallidum*; (3) To assess the putative role in sensory transduction of Mcp1 and three other methyl-accepting chemotaxis proteins of *T. pallidum*; (4) To implement a new combined genome- and invasin-based strategy to identify *T. pallidum* rare outer membrane proteins that may qualify as syphilis vaccine candidates; and (5) To continue to investigate mechanisms by which *T. pallidum* and its proinflammatory lipoproteins facilitate HIV transmission, with emphasis on examining the upregulation of CCR5, the HIV-1 coreceptor, on *T. pallidum*-activated immune cells. The pursuit of these aims will further our understanding of *T. pallidum* membrane biology relevant to syphilis pathogenesis, tissue dissemination, and vaccine development, as well as delineate the molecular constituents which induce salient inflammatory processes that culminate in clinical disease. The aims also seek to advance new strategies for utilizing surrogate genetic systems for *T. pallidum*, such as *E. coli* and *T. denticola*, as a means of elucidating the interrelationship between *T. pallidum* membrane biology and syphilis pathogenesis.

Grant: 2R01AI019146-18
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: OHMAN, DENNIS E PHD
MICROBIOLOGY:IMMUNO
EMISTRY
Title: ACTIVATION OF ALGINATE GENES IN PSEUDOMONAS AERUGINOSA
Institution: VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA
Project Period: 1982/09/01-2004/11/30

This proposal is designed to elucidate molecular mechanisms of pathogenesis in *Pseudomonas aeruginosa* strains infecting patients with Cystic Fibrosis (CF). Following the initial infection, *P. aeruginosa* undergoes mucoid conversion and acquires the ability to cause chronic, debilitating, and life-threatening disease. The mucoid phenotype is due to the overproduction of a capsule-like exopolysaccharide called alginate, which is associated with resistance to phagocytosis and new adherence properties. Adaptive mutations occur *in vivo* to derepress *algT*-encoded alternative sigma factor-22, which also controls a global stress response. This leads to the activation of a regulatory cascade that includes the overproduction of alginate. This proposal addresses the elucidation of this bacterial stress response during pulmonary infection in CF. We will define the mechanism of sigma-22 posttranscriptional regulation by MucABCD, which apparently forms a membrane complex that controls sigma-22 turnover by an unknown signal transduction mechanism. Sigma-22 controls the genes of a stress response regulon that is probably activated upon infection, and it is hyperactivated upon mucoid conversion. We will define the genes under sigma-22 control that are activated upon mucoid conversion to better understand the response system that is so highly expressed upon mucoid conversion. The sensors (or receiver modules) called KinB and FimS are probably responsible for detecting important environmental conditions during infection, and then transmit this information to their regulators, AlgB and AlgR, respectively. This in turn activates the expression of genes that are probably involved in *in vivo* survival. To better understand these sensor-regulator systems, we will define the roles of these sensors of the 2-component systems under sigma-22 control by elucidating the genes under their control. In addition, we have found that the *rpoS* encoded sigma factor sigma-S (or RpoS) controls alginate production, and so probably controls a step in the sigma-22 regulatory cascade. We will characterize the role of sigma-S in the alginate regulon and other genes it controls that are associated with the stress response during infection. The information gained from the analysis of this complex regulon, which is activated upon infection of the CF lung by *P. aeruginosa*, improve our understanding of this unique host-pathogen interaction and may lead to the development of successful therapy.

Grant: 2R01AI019782-14
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: BYRNE, GERALD I PHD
Title: IMMUNITY AND LATENCY TO CHLAMYDIAL INFECTIONS
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 1982/08/01-2004/11/30

Members of the genus Chlamydia, a group of obligate intracellular procaryotic pathogens, are important causes of human infectious diseases. Chlamydia pneumoniae recently has been implicated in the initiation and development of atherosclerosis. Chlamydia trachomatis is a major cause of preventable infectious blindness and the leading cause of bacterial sexually transmitted diseases (STD). Upper genital tract complications in females represents a significant women's health issue. Silent pelvic inflammatory disease (PID) can lead to tubal obstructive infertility. This serious disease will require extensive investigation to understand the pathogenic processes that cause irreparable damage of the reproductive tract in women during their child-bearing years. It is important to discern pathologic changes that accompany atherosclerosis, trachoma, PID and tubal obstructive disease as these events actually occur in infected people, but studies involving human populations do not lend themselves well to carefully controlled experimental conditions. Therefore we propose to continue our work using a variety of cell culture systems (human and murine) to study general features of persistent intracellular chlamydial growth which may be common to all chronic chlamydial infections and pursue the murine-C. trachomatis genital tract in vivo model to study the hypothesis that persistent chlamydiae may contribute to the development of upper genital tract disease. This hypothesis will be tested by building on our experience related to the effects of immune response regulated cytokines on chlamydial host cell activation that results in enhanced expression of chlamydial stress response proteins together with new information on the effects of stress response proteins on the disease process. We also will study how these fundamental events in the basic biology of chlamydiae relate to chronic disease as exemplified by upper genital tract infections in mice. The work plan will comprise 4 specific aims, 2 of which are intended to broaden our cell culture knowledge of persistent (stressed) chlamydial growth and 2 of which will apply this knowledge to an in vivo system. Results will lead to increased information concerning how the basic biology of chlamydiae directly impacts the disease process and the development of chronic chlamydial disease.

Grant: 2R01AI019844-17
Program Director: LANG, DENNIS R
Principal Investigator: MC CLANE, BRUCE A
Title: MECAHNISMS OF ACTION OF C PERFRINGENS EXTEROTOXIN
Institution: UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PA
PITTSBURGH
Project Period: 1982/07/01-2005/03/31

Clostridium perfringens enterotoxin (CPE) has now been conclusively identified as the virulence factor responsible for symptoms associated with several of the most common foodborne and nonfoodborne gastrointestinal (GI) illnesses of bacterial origin. The long term objective of this project is to fully understand the pathogenesis of CPE-associated GI diseases, including identification of the mechanism of action of CPE, and to identify strategies to prevent or treat these illnesses. To progress towards this goal, the following specific aims will be pursued in the next grant period: 1) evaluating the importance of claudins as CPE receptors for human intestinal cells through Northern analyses and "anti-receptor" antibody studies; if claudins are confirmed as important CPE receptors, claudin: CPE interactions will be explored by phenotyping a series of claudin random mutants for their ability to bind CPE and convey cytotoxicity, 2) identifying the eucaryotic protein constituents of CPE-containing small and large complexes by immunoblot and immunoprecipitation analyses; the importance of each eucaryotic complex protein for CPE action will then be dissected through antibody inhibition studies, 3) using site-directed mutagenesis to high-resolution map CPE functional regions, including the recently identified receptor-binding and large complex-forming regions of the toxin; results generated with these CPE mutants will then be interpreted within the context of the 3-D structure of CPE, and 4) dissecting the molecular pathogenesis of cpe-positive isolates by physical mapping of the cpe plasmid in nonfoodborne disease isolates, determining whether the cpe plasmid can be transferred to other C. perfringens isolates, evaluating whether the chromosomal cpe of food poisoning isolates is dn a mobilizable transposon, and determining whether two component regulatory systems and/or the exponential growth phase repressor Hpr play a role in regulating the sporulation-associated expression of CPE.

Grant: 2R01AI020624-17
Program Director: MILLER, MARISSA A.
Principal Investigator: HOOK, MAGNUS A
Title: ANALYSIS OF STAPHYLOCOCCUS AUREUS HOST INTERACTIONS
Institution: TEXAS A&M UNIVERSITY HEALTH SCIENCE COLLEGE STATION, TX
CTR
Project Period: 1989/12/01-2005/03/31

The ultimate goal of our studies is to understand in molecular terms how bacteria cause diseases. A bacterial infection can be regarded as a war between the microbe and the host where the bacteria's attempt to adhere to and colonize the host tissue represent the first battle in the campaign. For our model organism, *Staphylococcus aureus* tissue adherence is mediated by a sub-family of bacterial surface adhesins called MSCRAMMs. In previous work, we discovered the MSCRAMMs, cloned and sequenced several MSCRAMM genes and began characterizing the encoded proteins and their interactions with host components. These studies revealed amazingly sophisticated mechanisms of host tissue adherence designed to avoid inactivation by host defense systems. We hypothesize that the MSCRAMMs are in the first line of bacterial attachment and their molecular design makes them uniquely suited for this role. Consequently, we now propose a detailed molecular analysis of Staphylococcal surface proteins and their interactions with host components.

Grant: 2R01AI021150-15
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: SHAFER, WILLIAM M
Title: GONOCOCCI: GENETICS OF RESISTANCE TO PMN PROTEINS
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 1984/04/01-2005/03/31

DESCRIPTION: (Adapted from the application abstract) *Neisseria gonorrhoeae* is an obligate human pathogen and has the capacity to infect and cause disease at numerous sites. However, this capacity requires that gonococci resist antibacterial substances that naturally bathe mucosal surfaces or become available due to inflammation. The emphasis of this project is to understand the mechanism(s) used by gonococci to resist antibiotic-like substances that bathe certain mucosal sites. The *mtr* (multiple transferable resistance) and *far* (fatty acid resistance) loci contain operons that encode efflux pump proteins that export structurally diverse antibacterial hydrophobic agents (HAs), including bile salts, fatty acids, lysosomal proteins and antibiotics. The *MtrCDE* and *FarAB* proteins belong to families of bacterial proteins that form efflux pumps that remove structurally diverse antimicrobial agents from either the periplasm or cytoplasm. Expression of these efflux pump operons are subject to both negative and positive transcriptional control systems. For instance, the *MtrR* protein down-regulates expression of the *mtrCDE* operon through its capacity to bind to the *mtrCDE* promoter and this results in enhanced susceptibility of gonococci to certain HAs. Conversely, expression of the *farAB* operon, which encodes an efflux pump that exports long-chained fatty acids with potent antigonococcal activity, seems to be dependent on *MtrR*. Through the use of modern techniques in microbial genetics, molecular biology and biochemistry, we will determine the mechanisms by which *MtrR* exerts transcriptional control over these efflux pump operons and other gonococcal genes (Specific Aims 1 and 3). Expression of the *mtrCDE* operon can also be induced during exposure of gonococci to sub-lethal levels of HAs. This induction process requires a transcriptional activator, *MtrA*, that belongs to the *AraC/XylS* family of DNA-binding proteins. The mechanisms by which *MtrA* exerts its control over gonococcal gene expression will be determined (Specific Aim 2). Dr. Shafer's group has recently identified a novel protein (*MtrF*) that seems to act as a component of the *mtrCDE*-encoded efflux pump. *MtrF* counterparts exist in several other bacteria but their function has yet to be determined. Given its apparent wide-spread distribution, they will determine its role in efflux pump activity (Specific Aim 4). The results from these studies will advance our knowledge regarding how gonococci and other pathogens resist antimicrobial agents at mucosal surfaces, antibiotics used in therapy of bacterial diseases, and topical microbicides that have been proposed for use to prevent sexually transmitted diseases.

Grant: 2R01AI021242-16A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: WALKER, DAVID H
Title: SPOTTED FEVER RICKETTSIAL ANTIGENS
Institution: UNIVERSITY OF TEXAS MEDICAL BR GALVESTON, TX
GALVESTON
Project Period: 1992/09/30-2004/08/31

DESCRIPTION (Adapted from the Applicant's Abstract): The long-term objectives of this project are to identify the molecular components of spotted fever group (SFG) rickettsiae that stimulate protective immunity and the rickettsicidal immune mechanisms that effectively clear the rickettsial infection. Rocky Mountain spotted fever, the most severe and widespread rickettsiosis in the US, continues to kill more than 5% of patients because of diagnostic difficulty and lack of a means for protection. Numerous SFG rickettsioses are prevalent in other parts of the world, and the immune mechanisms are very similar for the resurgent epidemic louse-borne typhus and for murine typhus. The health impact of this research is that it will establish the knowledge and principles to enable the production of effective vaccines against rickettsial diseases. The specific aims of this project will be to: 1) define the dominant CD8 T-lymphocyte epitopes of rOmpA and rOmpB of *Rickettsia conorii* at the oligopeptide level and identify the epitopes processed intracellularly and presented on the cell membrane by endothelial cells for activation of CD8 T-lymphocytes, and evaluate these dominant CD8 T-lymphocyte epitopes for protection in the appropriate mouse model(s); 2) map the B-lymphocyte epitopes of rOmpA and rOmpB and determine the role of antibodies in immune protection against *R. conorii*; and 3) determine the role of the T-lymphocyte targeting chemokines (IP-10, Mig, and fractalkine) produced by rickettsia-infected endothelial cells in the chemotaxis, localization, and activation of effector CD8 T-lymphocytes. The peptides that activate purified CD8 T-lymphocytes will be identified using both macrophages and endothelial cells as antigen presenting cells initially in order to validate the approach using endothelial cells transfected with a eukaryotic expression vector expressing rOmpA and rOmpB peptides. Mapping of the CD8 T-cell epitopes will employ a strategy of subcloning overlapping DNA fragments followed by selection of overlapping synthetic peptides. The B-lymphocyte epitopes of rOmpA and rOmpB will be determined using recombinant peptides of *R. conorii* rompA and rompB and finally overlapping synthetic oligopeptides. Conformational epitopes will be determined using recombinant peptides from a random peptide library expressing minotopes. The epitopes that stimulate CD8 T-lymphocytes and antibody production will be evaluated for their ability to stimulate protective immunity in mice and by passive transfer of CD8 clones and monoclonal antibodies. The remarkable cross protection between the divergent SFG rickettsiae, *R. conorii* and *R. australis*, will be exploited in our strategies to identify the protective epitopes. Because the immune control of rickettsial infection occurs mainly inside infected cytokine-activated endothelial cells which are targets of CTL activity and adjacent to which CD8 T-lymphocytes have infiltrated, the role of chemokines in 3 particular endothelial cell-derived lines will be determined in the chemotaxis, localization, and activation of the effector CD8 T-lymphocytes using a model of the retinal vascular bed in vivo and endothelial cell cultures. The overall outcome of this project will be the expansion of

Includes Research Project Grants (RPGs)
Excludes clinical trials

our knowledge of the most important, insufficiently understood elements of rickettsial immunity: CD8 T-lymphocytes, antibodies, stimulating epitopes at the level of synthetic oligopeptides, and the mechanisms of chemotaxis and activation of CD8 T-lymphocytes in the rickettsia-infected lesions.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2R01AI021451-14
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: LORY, STEPHEN PHD
MICROBIOLOGY: BACTERIOLOGY
Title: P. AERUGINOSA VIRULENCE DETERMINANTS
Institution: HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA
Project Period: 1984/08/01-2005/08/31

DESCRIPTION (Adapted from applicants' abstract): The ability to secrete proteins to their surrounding medium is an essential virulence characteristic of most pathogenic bacteria. The major barrier to extracellular protein secretion in gram-negative bacteria is the outer membrane. Accordingly, these bacteria have evolved elaborate pathways for extracellular targeting of proteins. One such protein export mechanism, referred to as the type II secretion pathway is responsible for secretion of proteins that have been previously localized into the bacterial periplasm. The type II pathway which functions in the human pathogen *Pseudomonas aeruginosa*, is responsible for the secretion of a number of important virulence factors. The machinery of the type II secretion has been previously shown to consist of 12 proteins, encoded by the xcp genes. Additional genes that encode homologues of the Xcp proteins have been identified primarily through the *P. aeruginosa* genome-sequencing project. The objectives of this proposal are to study the function of the machinery of extracellular protein secretion by defining the structure of the assembled secretion organelle in the cell envelope and to initiate a program for the identification of synthetic chemical compounds that target this pathway. Three specific aims are proposed to accomplish these objectives. First, an interactive map of all of the components of the type II secretion machinery will be generated, utilizing a combination of approaches including chemical cross-linking, analysis of protein-protein interactions in yeast and a bacterial two hybrid systems and through the use of phage display technology. The second aim will specially address the structure and function of the outer membrane components of the secretory apparatus. Finally, aided by the use of expression microarray technology, reporter strains will be engineered which will be used as sensitive indicators of a secretion block. These strains will be then used to screen libraries of chemical compounds which specifically inhibit the type II secretion pathway. If successful, the research proposed in this application should provide important new insights into the function of a secretion mechanism which is present in a large number of bacterial pathogens and could lead to the development of potentially new categories of antimicrobial agents.

Grant: 2R01AI021628-15
Program Director: KLEIN, DAVID L
Principal Investigator: MURPHY, JOHN R PHD
Title: DIPHTHERIA TOX REPRESSOR: GENETIC & STRUCTURAL ANALYSIS
Institution: BOSTON MEDICAL CENTER BOSTON, MA
Project Period: 1984/05/01-2004/11/30

The diphtheria toxin repressor, DtxR has been cloned from genomic libraries of *Corynebacterium diphtheria*. DtxR folds into a N-terminal (M1-G136) and C-terminal (R161-L226) domain structure which are connected by a random coil linker (E137-N160). The N-terminal domain of DtxR contains the helix-turn-helix DNA binding motif, the primarily and ancillary metal ion-binding domain, and a protein-protein interaction region. Until recently, the structure of the C-terminal domain of DtxR (Qiu et al., 1997) and NMR spectroscopy of the C-terminal peptide DtxR (C102D) bind to opposite sides of the diphtheria tox operator following a metal-ion triggered subunit "caliper-like" movement which aligns the HTH motif in the major groove and a helix-to-coil transition of the N-terminal six amino acids (White et al., 1998). Apo-DtxR is an inactive monomeric form; however, upon addition of activating metal ions, DtxR undergoes a conformational change to an active dimeric structure. During the last grant period we have developed an extremely powerful positive genetic selection system (PSDT) that links chloramphenicol resistance to a functional DtxR:tox operator genetic circuit. Using the PSDT system we have isolated the first self-activated iron-independent mutants of DtxR. The simplest mutant in this new class, DtxR (E175K) carries a single point mutation in its SH3-like domain. Given the apparent flexibility of this C-terminal SH3-like domain we propose that the epsilon-amino moiety of K175 in this mutant is able to insert into the primary metal ion-binding site and serves as a surrogate for iron in the activation of repressor activity. Based upon this hypothesis, we have screened a peptide library for peptides capable of activating wild type DtxR. This search has yielded the first iron-mimetic peptides capable of activating wild type DtxR. This search has yielded the first iron-mimetic peptides capable of activating wild type DtxR repressor activity in the absence of iron. The long term goals of this proposal are focused on the elucidation of the molecular events which modulate the conversion of inactive apo-DtxR to its active dimeric form, and the mechanism of peptide-mediated activation of repressor activity in the absence of iron. We anticipate that iron mimetic activations of the DtxR family of repressors may be prototypes of a new class of "antibiotic" that selectively repress iron-sensitive gene expression and thereby attenuate virulence.

Grant: 2R01AI022160-12A1
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: TAINER, JOHN A PHD
Title: ASSEMBLY AND STRUCTURE OF TYPE IV PILI
Institution: SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA
Project Period: 1985/07/01-2005/01/31

This renewal aims to characterize structure-function relationships for type IV pili fibers, which key virulence factors for pathogenic Gram-negative bacteria. Structural analyses for type IV pilin subunits will be integrated with electron microscopy (EM), fiber diffraction, and small angle x-ray scattering (SAXS) structures of native fibers via objective Fourier correlation methods. These proposed studies, which span atomic to subcellular resolutions, will focus upon type IV pili from *Neisseria gonorrhoeae*, the causative agent of gonorrhea. Successful methods and results on gonococcal pili will allow complementary structural and mutational studies on pili from *Pseudomonas aeruginosa*, the causative agent on deadly opportunistic nosocomial infections, and *Vibrio cholerae*, the causative agent of cholera, to define conserved and variable aspects of type IV pili. Key questions concerning pilus structure-function relationships will be addressed including whether the *N. gonorrhoeae* pilin fold is representative of all type IV pilins, how extreme antigenic variation avoids disrupting the pilin fold and fiber assembly, the nature and significance of post-translational modifications, structural changes associated with fiber formation, species-specific conservation of surface regions acting in target cell recognition and accessory protein binding, the structural chemistry controlling bundling, structural characteristics of immunodominant regions, and optimal approaches to the design of cross-species vaccines. Structural results and hypotheses will be experimentally tested by quantitative correlations among diffraction and electron microscopy results and by mutational analyses. The proposed integrated multi-disciplinary studies provide innovation in determining challenging fiber-forming protein structures and in bridging the huge resolution gap between protein crystal structures and EM image reconstructions of subcellular organelles. Overall, these structural and mutational results will promote integration of ongoing biochemical, immunobiological, genetic, and functional studies to decipher the structural chemistry governing pilus actions in pathogenicity: host cell surface attachment, twitching motility, bacteriophage absorption, modulation of transformation efficiency and toleration of extreme sequence variability while retaining structural integrity and flexibility. This understanding of pilus structure-function relationships has long-term potential applications for drug and vaccine design against major bacterial diseases now showing increasing antibiotic resistance and threats to public health.

Grant: 2R01AI022501-14
Program Director: BAKER, PHILLIP J.
Principal Investigator: LANE, ROBERT S PHD
Title: LYME DISEASE IN WESTERN USA--ECOLOGY AND EPIDEMIOLOGY
Institution: UNIVERSITY OF CALIFORNIA BERKELEY BERKELEY, CA
Project Period: 1985/09/01-2005/08/31

DESCRIPTION (Adapted from the Applicant's Abstract): The broad objectives of this research are to determine environmental correlates of natural foci of the Lyme disease (LD) spirochete, *Borrelia burgdorferi sensu stricto* (Bb ss) and closely related spirochetes in the Bb sensu lato (sl) complex in highly endemic areas of the Far West; to elucidate and model landscape ecologic and epidemiologic factors that place humans at elevated risk of exposure to Bb ss infection in meso- and macroscales by means of remote sensing and ground-truthing ecologic studies; and to evaluate intrinsic factors that may affect the reservoir competence of vertebrates or the vector competence of ticks for Bb sl. Environmental correlates of natural foci of LD spirochetes will be determined quantitatively by sampling populations of vector ticks and their small vertebrate hosts in relation to Bb sl infection and over 20 biotic and abiotic factors. Two categorical measures will be used to assess risk of exposure to nymphs of the primary vector tick, *Ixodes pacificus*, in leaf-litter habitats, an indirect measure (entomologic risk index) based on drag-sampling and direct measures of specific human behaviors (e.g., walking, sitting). A combination of field and laboratory methods will be employed to calculate the relative reservoir potential (Rs) of small mammals for Bb sl in deciduous woodlands. The prevalence of infection in free-living or host-fed ticks and their attendant vertebrates with Bb sl and two other emerging tick-borne disease agents (*Ehrlichia* spp.) Will be determined by tick xenodiagnosis and with standard microbiologic and genetic techniques. Bb sl isolates cultured from vector ticks, reservoir hosts, or skin lesions of patients with early-stage LD infection will be characterized genetically with several methods, and their phylogenetic relatedness to other genospecies of Bb sl will be determined by maximum parsimony analyses. Risk of human exposure to Bb-infected *I. pacificus* nymphs inhabiting all three climatic zones and seven major vegetational types of northwestern California (Mendocino County) will be modeled using satellite imagery, geographic information systems, and spatial statistical methods in conjunction with ground-verifying ecologic studies and dLD case surveillance data. Borreliacidal assays will be used to evaluate the potential role of complement in vertebrate-host sera (16 spp.) And anti-complement activity in saliva of human-biting vector ticks (2 spp.) As determinants of reservoir-host competence or vector competence for Bb sl or relapsing fever group spirochetes present in the western USA.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2R01AI022535-16
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: PIER, GERALD B PHD MICROBIOLOGY, OTS
Title: ACQUIRED IMMUNITY AND VACCINATION FOR P AERUGINOSA
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 1984/12/01-2005/07/31

DESCRIPTION (Adapted from the Applicant's Abstract): The major goals of this project are to understand basic aspects of virulence and host immunity to lipopolysaccharide (LPS)-smooth strains of *P. aeruginosa* that are major causes of hospital acquired pneumonia and community-acquired bacterial keratitis of the eye. The primary hypothesis to be evaluated is that effective immunity to *P. aeruginosa* will require the use of immunogens that elicit both antibodies and T cells that can kill extracellular and intracellular *P. aeruginosa*. In regard to immunity to the extracellular stage of pathogenesis, for many *P. aeruginosa* strains, effective humoral immunity requires antibodies that recognize highly variable subtype-specific epitopes on the LPS O side chain. Subtype epitope variability greatly increases the number of antigens that need to be incorporated into a vaccine that go beyond the 20 major LPS-based serogroups of *P. aeruginosa*. More problematic has been the difficulty in inducing such antibodies by LPS-O antigen based immunogens. Appropriate LPS-specific antibodies cannot readily be elicited with polyvalent vaccines because of antagonistic interactions among structurally-related immunogens and the host immune system, so newer antigen-delivery systems are needed. To address this need, the investigators are focusing on producing and testing live, attenuated *P. aeruginosa* vaccines that would be better immunogens for inducing LPS-specific protective antibody and also provide immunity to the recently documented intracellular phase of *P. aeruginosa* infection. *P. aeruginosa* enters epithelial cells via the cystic fibrosis transmembrane conductance regulator (CFTR) during the course of lung and eye infections, and epithelial cell ingestion has a significant impact on the organism's ability to cause infections. The major aim of the proposal will be to explore how attenuated vaccines that enter lung epithelial cells prior to clearance induce protective antibodies and T cells that recognize infected epithelial cells and kill the intracellular *P. aeruginosa*. Overall, the aims are designed to gain a better understanding of how to elicit protective immunity to *P. aeruginosa*, focusing on use of attenuated strains to provoke humoral and cell-mediated immunity (CMI) against both cytotoxic and noncytotoxic *P. aeruginosa* strains. Specific questions to be addressed include: 1) can attenuated strains of *P. aeruginosa* be safely produced that elicit humoral and cellular immune effectors that kill *P. aeruginosa* outside and inside of epithelial cells?; 2) can such vaccines elicit broadly-based acquired resistance to *P. aeruginosa* infection; and, 3) how is CMI elicited by attenuated vaccines and how does this immune effector contribute to resistance to infection? The results of this work would further our insights into *P. aeruginosa* pathogenesis, development of acquired immunity and the potential to develop effective reagents for active and passive immunization to augment the prevention and treatment of *P. aeruginosa* infections.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2R01AI023007-14
Program Director: TSENG, CHRISTOPHER K.
Principal Investigator: CROSS, TIMOTHY A PHD CHEMISTRY, OTHER
Title: CORRELATIONS--STRUCTURE DYNAMICS FUNCTIONS IN CHANNELS
Institution: FLORIDA STATE UNIVERSITY TALLAHASSEE, FL
Project Period: 1987/01/01-2005/02/28

Description: (Verbatim from the applicant's abstract) Single channel conductance measurements and high resolution structure and dynamics characterization from solid-state NMR will lead to structure-function and dynamics-function correlations for ion channels. Previous support through this grant has led to novel correlations for explaining the conductance specificity and efficiency in the monovalent cation selective channel, gramicidin A. Insights into cation binding, electrostatic reduction of potential energy barriers and Grotthus conductance has generated principles and predictions for other channels. Indeed, the realization that the selectivity filter region of the K⁺ channel from *Streptomyces lividans* is lined with backbone amide groups suggests that the gramicidin pore which is similarly lined is an important model system. Here we propose to study the KcsA channel from *Streptomyces* in collaboration with Chris Miller (Brandeis Univ.), who has overexpressed and reconstituted the channel in bilayers. We will also study the M2 protein from Influenza A virus, a H⁺ channel that has been overexpressed and reconstituted in our own lab thanks to a previous collaboration with Robert Lamb (Northwestern Univ.). And we will continue to search for fundamental explanations of conductance properties, such as channel flicker and open channel noise in the structure and dynamics of gramicidin A. The comparison of functional, dynamics and structural studies, all obtained from a lamellar phase lipid environment, is an important advantage for this approach. This work has broad implications in the field of ion channels through the elucidation of the molecular details for channel gating and blocking, and also in demonstrating a method for achieving high resolution structural and dynamic characterizations for membrane proteins in a lamellar phase lipid environment

Grant: 2R01AI023362-15
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: KRAUSE, DUNCAN C
Title: GENETIC ANALYSIS OF MYCOPLASMA PNEUMONIAE CELL ADHERENCE
Institution: UNIVERSITY OF GEORGIA ATHENS, GA
Project Period: 1986/09/01-2004/04/30

DESCRIPTION (Adapted from applicant's abstract): Mycoplasma pneumoniae is the leading cause of pneumonia in older children and young adults. Fundamental aspects of mycoplasma cell and molecular biology remain poorly understood, despite the impact of mycoplasmas on public health and agriculture. More effective means of prevention, control and resolution of infectious disease requires elucidation of the fundamental biological processes of the etiological agents involved. With the M. pneumoniae genome sequence complete, this will require continued pursuit and application of approaches to manipulate the mycoplasma genome in novel ways. M. pneumoniae colonization of the respiratory epithelium (cytadherence) is a complex process that includes proteins directly involved in receptor binding, as well as those having an accessory role involving assembly of the attachment organelle. The focus of this proposal is the structure, function and regulation of several mycoplasma proteins having very different roles in the adherence process. The adhesin P1 is concentrated at the attachment organelle, and the applicant will investigate by deletion analysis and green fluorescent protein fusions, the domains of P1 essential for trafficking to the attachment organelle and association with the mycoplasma cytoskeleton. Protein P30 is required for cytadherence, but loss of P30 is also associated with a developmental defect. This phenomenon will be explored in more detail, focusing in particular on nucleoid condensation and partitioning in P30 mutants, as well as identification of domains important in P30 function and subcellular localization. Finally, HMW1 is required for development of the attachment organelle and for P1 trafficking to this structure. The C-terminus of HMW1 is essential for function and is also targeted for accelerated proteolysis in hmw2 mutants. Structure-function studies will be pursued with HMW1, including identification of the target site(s) for proteolysis and analysis of the role of proteolysis in controlling HMW1 function in cell development.

Grant: 2R01AI024145-13
Program Director: LANG, DENNIS R
Principal Investigator: BLASER, MARTIN J. MD INTERNAL
MED:INFECTIOUS DISEASE
Title: CELL-SURFACE PROTEINS IN CAMPYLOBACTER FETUS VIRULENCE
Institution: VANDERBILT UNIVERSITY NASHVILLE, TN
Project Period: 1989/09/01-2005/08/31

DESCRIPTION (Adapted from the Applicant's Abstract): *Campylobacter fetus* is a pathogen of humans and animals. All wildtype *C. fetus* strains possess regular, paracrystalline high molecular weight (97, 127, or 149kDa) surface layer proteins (SLPs) that are critical for virulence. The SLPs inhibit complement binding, which leads to both serum and phagocytosis resistance, and they undergo antigenic variation. *C. fetus* cells possess 8 or 9 homologs of *sapA*, which encodes a 97 kDa SLP. The homologs share multiple areas of homology with each other, are clustered on the bacterial genome, and together with an invertible region (IR) of 6kb form the *sap* locus. The investigators previously showed that the DNA rearrangement (inversion) plays a major (if not exclusive) role in the antigenic variation, and that mutation of *recA* removed all detectable variation. They now have developed animal models to examine SLP variation, have shown that antigenic variation due to *sap* locus recombination occurs in *recA* strains, and have moved the IR onto the *E. coli* chromosome. The first aim of the proposed work is to identify the recombination pathways that permit the *sap* locus inversion. Their hypothesis is that the inversion depends on conserved recombination genes including those in the RecBCD, RecE, and RecF pathways. Alternatively, they might find that *C. fetus* has species-specific recombination pathways. The ultimate goal will be to define and mutate the recombination genes in *C. fetus* and examine the phenotypes of mutants. Their second aim is to define the structural features present in the *sapA* homologs that are required for recombination to occur. They propose to complete the map of the *sap* locus and to create mutants that will permit assessment of the necessary structural features. As part of this goal, they plan to identify the sites of double strand breaks that are the initial sites for DNA strand exchange. The third aim is to examine *sap* rearrangement in vivo in mice. After establishing the model, they can examine the role of host responses in directing the evolution of antigenic variation, and the fitness of defined mutants. Finally, the data generated can be used to develop a mathematical model of antigenic variation that focuses on the relationship between microbial recombination generating diversity and host selection determining the direction of change.

Grant: 2R01AI024452-10A2
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: STEIN, DANIEL C PHD
Title: LIPOOLIGOSACCHARIDE BIOSYNTHESIS IN NEISSERiaceae
Institution: UNIVERSITY OF MARYLAND COLLEGE PK COLLEGE PARK, MD
CAMPUS
Project Period: 1988/09/30-2005/05/31

Description (adapted from the applicant's abstract): *Neisseria gonorrhoeae* is responsible for over 1 million cases of gonorrhea each year in the United States and the total health care costs associated with treating gonorrhea, and complications that arise from infections caused by this organism exceed 1 billion dollars per year. An effective vaccine would dramatically reduce the associated health care costs. This proposal focuses on elucidating the genetic mechanisms responsible for the expression of one of the organism's principal surface antigens, lipooligosaccharide (LOS). In the work to be described, I will identify by gene cloning and genetic complementation techniques, genes required for the LOS biosynthesis. I will characterize these genes by DNA sequence analysis, define the biochemical properties of the gene products and determine their role in the biosynthetic process. Since the gonococcus can vary its LOS, depending on the host and the local environment, studies on the genetic regulation of its synthesis are warranted. Stable LOS-producing strains are needed to allow us to dissect the role of LOS in the disease process. I will construct a series of genetically defined LOS mutants that express defined LOS structures. These strains will also allow us to study the interaction of the expression of this molecule with other cell surface components. Understanding how the expression of LOS is regulated will allow us to design experiments to test the role of each cell surface component in the disease process. By understanding the relationship between disease and the expression of a specific surface component, we can design vaccines that can prevent the disease in specific demographic groups.

Grant: 2R01AI027913-12
Program Director: KLEIN, DAVID L
Principal Investigator: TUOMANEN, ELAINE I
Title: BIOACTIVITIES OF PNEUMOCOCCAL CELL WALL IN MENINGITIS
Institution: ST. JUDE CHILDREN'S RESEARCH HOSPITAL MEMPHIS, TN
Project Period: 1989/06/01-2005/01/31

The pneumococcus remains the cause of meningitis with the greatest morbidity and mortality in children and older adults. This pattern persists despite the use of antibiotics of exceptionally rapid bactericidal activity. Over the past 10 years of this proposal we have sought to understand the biochemical basis of the inflammatory response to pneumococci in the subarachnoid space. We established that the pneumococcal cell wall is a library of inflammatory components which incites the cytokine, coagulation and arachidonate cascades and directly injures endothelial cells of the blood brain barrier. Further, we established that the release of cell wall during antibiotic-induced death engenders a dramatic host response that is responsible for serious injury to host tissues. This provided a rationale for use of agents like dexamethasone that can act as partner drugs with antibiotics to selectively control the injurious components of the host defense response. The current proposal seeks to determine the molecular details of the mechanism of pneumococcal invasion into brain and how neuronal cells are killed during meningitis. Blocking information decreases some sequelae of infection but does not appear to be sufficient in controlling neuronal loss, particularly for pneumococcal disease. Over half of the current survivors of this infection still have major permanent sequelae. Understanding this process will allow design of agents to specifically attenuate these ongoing losses. We propose to apply our expertise in the identification and characterization of pneumococcal surface components, to map the process of transcytosis across the blood brain barrier. We will identify the pneumococcal components involved, specifically focusing on CbpA. This protein is required for pneumococcal invasion. Secondly, we will characterize the process of pneumococcal translocation in terms of the intracellular vesicle and the signaling process. Involvement of the PAF receptor that binds pneumococci and sIgA that ligates CbpA in actual translocation will be determined. Finally, we will investigate preliminary evidence that upon inhibition of apoptosis suggest this is an important contributor to sequelae. The detailed mechanism appears to be novel and will potentially instruct cell biology as well as pathogenesis.

Grant: 2R01AI029952-09
Program Director: RUBIN, FRAN A.
Principal Investigator: WESSELS, MICHAEL R
Title: IMMUNE RESPONSE TO THE GROUP A STREPTOCOCCAL CAPSULE
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 1991/07/01-2005/06/30

Description (Adapted from applicant's abstract): A global increase in invasive group A Streptococcus (GAS) disease (bacteremia, necrotizing fasciitis, and streptococcal toxic shock syndrome) that began in the 1980's has continued through the present decade and has focused attention on investigation of mechanisms of GAS pathogenesis. During the previous funding period, work in their laboratory and others further documented the central role of the hyaluronic acid capsular polysaccharide in GAS virulence in experimental models of local and systemic infection. These studies showed that the capsule interferes with the phagocytic killing, prevents internalization of GAS by epithelial cells, modulates adherence mediated by other GAS surface molecules, and acts as a ligand for attachment of GAS to CD44 on pharyngeal keratinocytes. Despite these other advances, it remains undefined how the capsule or other virulence determinants control the processes of tissue invasion and persistent colonization in the host. During the next funding period, their objectives are to define the role of the hyaluronic acid capsule in invasion of GAS from an epithelial surface to deep tissue, to characterize the effects of capsule on intracellular trafficking of GAS in epithelial keratinocytes, and to determine how regulation of capsule expression in vivo contributes to pathogenesis of GAS infection. The proposed experiments will make use of primary keratinocyte cultures and a model system simulating intact human skin in conjunction with confocal fluorescence microscopy in order to study the tissue and cell biology of GAS translocation through human skin, the phenomenon of persistence within cells, and the regulation of capsule expression at various phases of the infection process. Results of these studies will elucidate the basic pathogenic mechanisms involved in GAS disease and may suggest strategies for intervention.

Grant: 2R01AI030162-09A1
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: BARBIERI, JOSEPH T
Title: ACELLULAR VACCINES AGAINST BACTERIAL PATHOGENS
Institution: MEDICAL COLLEGE OF WISCONSIN MILWAUKEE, WI
Project Period: 1990/07/01-2005/06/30

Pseudomonas aeruginosa is a ubiquitous Gram-negative opportunistic pathogen of compromised patients. Especially susceptible are individuals inflicted with severe burn wounds, surgery, eye complications, and cystic fibrosis. *P. aeruginosa* pathogenesis is facilitated by its ability to grow in hospital environments and its intrinsic resistant to antibiotics. *P. aeruginosa* produces a number of virulence determinants, which are either cell-surface components or secreted. The goal of this proposal is to define the role of exoenzyme S (ExoS) in *P. aeruginosa* pathogenesis. Recent studies have shown ExoS to be a bifunctional type-III secreted cytotoxin. Expression of the amino terminus of ExoS in eukaryotic cells stimulates actin reorganization, which involves small-molecular-weight GTPases of the Rho subfamily. Expression of the carboxyl terminus of ExoS, the ADP-ribosyltransferase domain, inhibits the activation of Ras by its guanine nucleotide exchange factor. Rho and Ras are molecular switches, which control numerous cellular processes, including wound healing, tissue regeneration, phagocytosis, and T cell activation. This proposal will address the molecular basis for the inhibition of signal transduction by ExoS, by determining the roles that ADP-ribosylation and cytoskeleton rearrangement play in the pathogenesis of *P. aeruginosa*. The hypothesis is that ExoS allows *P. aeruginosa* to establish an initial site of infection, through the inhibition of Ras- and Rho- mediated signal transduction pathways, which are essential for cell proliferation. Understanding the molecular properties of ExoS should provide insight into the development of vaccines and therapeutics to prevent the clinical manifestations of *P. aeruginosa* infections. The specific aims of this proposal are to: define the biochemical and cellular properties of ExoS and ExoT, measure in vitro and in vivo ADP-ribosylation of eukaryotic proteins by ExoS, and determine the molecular basis for actin reorganization elicited by ExoS.

Grant: 2R01AI031338-09
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: HORWITZ, MARCUS A MD
Title: DEVELOPMENT AND TESTING OF NEW TUBERCULOSIS VACCINES
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 1991/04/01-2005/08/31

DESCRIPTION: (Adapted from Applicant's Abstract) Mycobacterium tuberculosis, the primary agent of tuberculosis, infects one-third of the world's population and kills 3 million people annually, making it the world's leading cause of death from a single infectious agent. It is a leading cause of disease and death in AIDS patients, particularly in the developing nations of the world. The rapid global emergence of strains resistant to the major antibiotics used to treat tuberculosis poses a serious threat to public health. The highest priority in the fight against tuberculosis is the development of a vaccine that is more efficacious than the current vaccine - BCG. A vaccine more potent than BCG would have an impact on human health greater than virtually any other conceivable development in the fight against infectious diseases. Studies from this laboratory completed under the current grant established the importance of major extracellular proteins of M. tuberculosis in inducing both cell-mediated and protective immunity in the guinea pig model of pulmonary tuberculosis, a highly susceptible species that develops disease remarkably similar to human tuberculosis. Studies under the current grant also succeeded in developing technology for high level expression and secretion in native form of major M. tuberculosis extracellular proteins in a nonpathogenic rapidly growing heterologous host, allowing isolation and purification of 100 mg quantities of recombinant M. tuberculosis extracellular proteins for vaccine studies. In this grant application, we seek to build on the knowledge and experience gained in previous studies to develop a vaccine more potent than BCG in the highly relevant guinea pig model. We seek to develop and test live recombinant vaccines including recombinant BCG expressing major M. tuberculosis extracellular and cell-associated proteins and new non-live particulate vaccines formulated as liposomes and microspheres.

Grant: 2R01AI032011-09
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: HANSEN, ERIC J PHD
Title: EXTRACELLULAR VIRULENCE FACTORS OF HAEMOPHILUS DUCREYI
Institution: UNIVERSITY OF TEXAS SW MED DALLAS, TX
CTR/DALLAS
Project Period: 1992/01/01-2005/06/30

Description (Adapted from the applicant's abstract): Chancroid, a ulcerogenital disease caused by the fastidious gram-negative bacterium *Haemophilus ducreyi*, is one of the least understood sexually transmitted diseases. The association between genital ulcer disease and transmission of the human immunodeficiency virus makes control and prevention of chancroid a public health concern. This research project involves investigation of two sets of *H. ducreyi* proteins which have in common the fact that they are released into culture supernatant fluid and have the potential to affect the host-parasite interaction in chancroid. The first set of protein are two very large macromolecules, designated LspA1 and LspA2, which contribute to the ability of *H. ducreyi* to resist killing by normal human serum. In addition, LspA1 affects virulence expression by *H. ducreyi* in an animal model independent of its involvement in serum resistance. The other set of proteins is encoded by the *cdtABC* gene cluster and comprise the cytolethal distending toxin (CDT) which has cytotoxic activity in vitro against human epithelial cells, human keratinocytes, and human T-cells. In the first Specific Aim, the PI will determine the molecular basis for how LspA1 affects virulence expression by *H. ducreyi* and how both LspA1 and LspA2 affect serum resistance of this pathogen. The second Specific Aim will involve characterization of the mature LspA1 and LspA2 proteins and investigation into whether the LspB protein effects their release from the *H. ducreyi* cell. In the third Specific Aim, the PI will identify the *H. ducreyi* gene product(s) which regulates expression of the LspA1, LspA2 and the LspB proteins. The fourth Specific aim entails the determination of the composition of the *H. ducreyi* CDT. The fifth and final Specific Aim will evaluate the LspA1, LspA2 and CDT proteins for their ability to induce immunity against *H. ducreyi* in an animal model.

Grant: 2R01AI032414-08
Program Director: LAUGHON, BARBARA E.
Principal Investigator: WHALEN, CHRISTOPHER C MD
Title: IMPACT OF TUBERCULOSIS OF HIV INFECTION IN UGANDA
Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH
Project Period: 1992/07/01-2004/08/31

DESCRIPTION: (Adapted from Investigator=s Abstract) The World Health Organization estimates that 10 million HIV-infected individuals will die of tuberculosis (TB) during the decade of the 1990's. Although HIV-infected patients with TB respond to effective antituberculous therapy, their prognosis remains poor. Early deaths are often attributable to TB, but later deaths result from complications of HIV infection other than tuberculosis. Recent epidemiologic observations indicate that TB reduces survival and increases the rate of opportunistic infections in HIV-infected patients. Mounting evidence from immunologic and virologic studies supports the concept of co-pathogenesis in which immune activation triggered by tuberculosis, and mediated by cytokines such as tumor necrosis factor-alpha (TNFa), stimulates viral replication, increases viral load, and accelerates HIV infection.

Grant: 2R01AI032493-08
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: SO, MAGDALENE Y PHD
MICROBIOLOGY: BACTERIOLOGY
Title: INTRACELLULAR SURVIVAL MECHANISMS OF NEISSERIA
Institution: OREGON HEALTH & SCIENCE UNIVERSITY PORTLAND, OR
Project Period: 1995/01/01-2004/12/31

DESCRIPTION (Adapted from the Applicant's Abstract): To initiate disease, the pathogenic *Neisseria* must invade non-ciliated cells of the mucosal epithelium, transcytose across the cell and exit into the subepithelial stroma. Cell culture and human volunteer studies demonstrate the *N. gonorrhoeae* have extended contact with its host, on the mucosal surface, and within cells. The Principal Investigator has been studying an important aspect of the intracellular life cycle of *Neisseria*: how the bacteria avoid lysosome killing. Their experiments demonstrate that the secreted *Neisseria* type 2 IgA1 protease is important for intracellular survival, as an iga mutant fails to replicate intracellularly. The protease also cleaves LAMP1 at its IgA1-like hinge, thereby accelerating its degradation and reducing its steady state levels in infected cells. LAMP1 is an integral lysosomal membrane glycoprotein which has been hypothesized to play a role in protecting the lysosomal membrane from degradation by its resident acid hydrolases. The Principal Investigator's results are consistent with this hypothesis. They indicate that infection results in multiple changes to lysosomes, as judged by decreased levels of several lysosomal constituents. They also show that the IgA1 protease is indirectly involved in these reductions. In this proposal, the Principal Investigator will continue her studies on *Neisseria* intracellular survival. She will determine how IgA1 protease reaches LAMP1 compartments, determine whether other secreted *Neisseria* proteases are capable of hydrolyzing LAMP1, identify the LAMP1 cleavage sites for the IgA1 protease, determine whether the protease is important for transcytosis and determine whether *Neisseria* escape the phagosome. Her studies should shed light on the function of the *Neisseria* protease(s) in intracellular survival. They may also help to determine whether the IgA1 protease is a worthwhile target for a gonococcal vaccine. As many other bacterial pathogens secrete proteases of unknown function with similar specificities for human IgA1, her studies may shed light on the function of a large class of bacterial proteases.

Grant: 2R01AI033096-10
Program Director: LANG, DENNIS R
Principal Investigator: NATARO, JAMES P
Title: VIRULENCE IN ENTEROAGGREGATIVE E COLI
Institution: UNIVERSITY OF MARYLAND BALT PROF SCHOOL BALTIMORE, MD
Project Period: 2000/08/01-2005/07/31

Enterotoxigenic Escherichia coli (EAEC) is an increasingly recognized pathogen of human diarrhea. This organism has been implicated in sporadic diarrhea in developing and industrialized countries, in the persistent diarrhea syndrome in AIDS patients and children in the developing world, in traveler's diarrhea, and in various diarrheal outbreaks. The PI discovered this pathotype of diarrheagenic E. coli and has been the leader in describing the pathogenesis and epidemiology of this organism. This is a competing continuation of our fundamental work on the pathogenesis of EAEC infection. Overall, our objectives are to advance knowledge of EAEC pathogenesis, to better define true EAEC pathogens, to refine diagnostic methods and to identify protective immunogen. The three aims of this proposal seek to extend the most important and promising aspects of the work funded under the current award. Aim 1: Characterization of EAEC Adhesion-Aggregation is the defining characterization of EAEC. Our observations suggest that aggregative adherence (AA) is a prelude to biofilm formation, which occurs in vivo and which can be modeled in vitro. In this aim, we will further elucidate fundamental aspects of EAEC adherence. Aim 2: The Regulation of EAEC virulence- AggR is a highly prevalent and conserved activator of AAF expression. However, nothing else is known of the regulation of EAEC virulence. Beginning with AggR, we will expand our studies of EAEC gene regulation. Aim 3: Reconstructing EAEC-We will use in vitro organ culture and T84 cell models of EAEC infection to answer the question: what genes are necessary and sufficient to confer the effects that we observe? The work under this award will greatly advance the current state of knowledge of EAEC and will result in the identification of pathogenetic mechanisms, of diagnostic reagents and in vaccine candidates.

Grant: 2R01AI033170-08
Program Director: MILLER, MARISSA A.
Principal Investigator: MOBASHERY, SHAHRIAR PHD
Title: RESISTANCE TO BETA-LACTAM ANTIBIOTICS
Institution: WAYNE STATE UNIVERSITY DETROIT, MI
Project Period: 1992/08/01-2005/06/30

DESCRIPTION (from the applicant's abstract): The catalytic function of beta-lactamases is the primary mechanism of bacterial resistance to beta-lactam antibiotics. A multidisciplinary approach has been outlined for the study of beta-lactamases, which builds on the mechanistic findings from the principal investigator's laboratory in the current cycle of funding. Four Specific Aims are outlined. Specific Aim 1 details the plans for cloning, expression, large-scale production, crystallization and characterization of the mechanism of action of the oxo-10 beta-lactamase from *Pseudomonas aeruginosa*. This enzyme is a prototypic member of the class D of beta-lactamases, which are understood the least currently. This enzyme is the parent enzyme for a number of expanded-spectrum beta-lactamases, which have been identified clinically recently. Specific Aim 2 describes the plans for investigation of the inhibitor-resistant TEM ("IRT") beta-lactamases. A subset of beta-lactamases is being identified since 1992 that resist inhibition by clinically used inhibitors. Four of these enzymes carry individually single mutations of mechanistic consequence for the inhibitor-resistant trait. These enzymes will be investigated in mechanistic and crystallographic efforts to elucidate their catalytic properties. Specific Aim 3 proposes a series of experiments in development of inhibitors for beta-lactamases that inhibit more than one class of these enzymes. Specific Aim 4 details research in elucidating the mechanistic and structural aspects for turnover of imipenem, a clinically important beta-lactam antibiotic, by the common class A TEM-1 beta-lactamase. This system exhibits certain desirable characteristics that make it amenable to structural analysis at every step of the catalytic process for turnover. These efforts collectively will help define the dynamic system of random mutation in the resistance genes and the process of selection that have given considerable diversity to these resistance enzymes in clinical settings.

Grant: 2R01AI033505-07A1
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: REST, RICHARD F PHD
MICROBIOLOGY: BACTERIOLOGY
Title: SIALYLATION OF THE PATHOGENIC NEISSERIA
Institution: MCP HAHNEMANN UNIVERSITY PHILADELPHIA, PA
Project Period: 2000/09/01-2005/08/31

Description (Adapted from applicant's abstract): Although *Neisseria gonorrhoeae* (Ng) and *Neisseria meningitidis* (Nm) cause very different diseases, they share the ability to covalently add sialic acid to their lipooligosaccharide (LOS). Sialylation of LOS leads to decreased association with host cells, and to resistance to phagocytic killing and serum killing. Therefore, sialylation is considered a major virulence factor. However, Ng and Nm are found within host cells during disease. In addition, they are not exposed to serum components during initial infection or carriage. Thus, there are times or conditions when the neisseria either needn't be sialylated (because it would be a waste of energy) or may not want to be sialylated (because they want to enter host cells). Thus, there is an apparent paradox. One way to explain this is to suggest that LOS sialylation is regulated by environmental signals in vivo. Dr. Rest has observed that Ng express more constitutive stase than Nm, and that the two organisms have very different DNA sequences upstream of their respective stase (*lst*) genes, which leads to differential expression at the transcriptional level. Also, Ng and Nm express up to 10 times or more stase activity when grown in broth than when grown on plates. Stase expression is also regulated by the absence of iron or the presence of nitrite. He would like to further define the regulation of stase expression in Ng and Nm, in vitro and in vivo. Toward this goal, he proposes the following specific aims: 1. Investigate the expression of stase by Ng and Nm in vitro. A. Perform mutational and deletional analyses. B. Investigate expression in various Ng and Nm regulatory mutants, including PilA/PilB, Fur, FNR, NarL/NarX, BasS/BasR, DegS-DegU and IHF. C. Determine if the newly defined 105 bp repeat is involved in stase expression. D. Determine the role of the above systems in regulation of stase expression by environmental signals. 2. Investigate the expression of stase by Ng and Nm in vivo. A. Determine stase expressed by Ng or Nm in contact with human epithelial or endothelial cells, neutrophils or serum. Investigate the role of extracellular vs intracellular residence. B. Use the regulatory mutants mentioned in Aim 1 as tools to investigate the mechanisms of in vivo *lst* regulation. 3. Determine whether stase is expressed on the outer or inner surface of the outer membrane of Ng and Nm. 4. Investigate whether LOS sialylation modulates serum resistance and association with host cells by DGI (disseminated gonococcal infection; stable serum resistant) strains.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2R01AI034436-06A2
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: CONNELL, NANCY D PHD
Title: NUTRIENT TRANSPORT MECHANISMS IN MYCOBACTERIA
Institution: UNIV OF MED/DENT NJ NEWARK NEWARK, NJ
Project Period: 1993/07/01-2005/05/31

DESCRIPTION (Adapted from the Applicant's Abstract): Mycobacteria are extremely important human pathogens. Their unusual cell wall structure and slow growth rate make them difficult to study from the standpoint of basic bacterial physiology. Mycobacteria have evolved into facultative intracellular parasites, capable of surviving within the phagocytic vacuole of the macrophage. It is likely that the ability of mycobacteria to acquire nutrients within the macrophage vacuole is tightly linked to intracellular survival and, therefore, to virulence. Knowledge of nutrient transport mechanisms for virulent mycobacteria will contribute directly to the design of novel therapeutic strategies and the development of new vaccines. The PI has isolated several mutants of *Mycobacterium bovis* BCG and *M. tuberculosis* which are deficient in transport of amino acids and peptides. In this competing continuation proposal, the PI will use classical and molecular genetic techniques and macrophage infection technology to address four specific aims: (1) to isolate and/or construct mutants of BCG and *M. tuberculosis* defective in transport and metabolism of arginine and oligopeptides; (2) to characterize transport of substrates by mutant and wild type BCG and *M. tuberculosis*; (3) to isolate and characterize transport regulatory mutants of BCG and *M. tuberculosis*; and (4) to examine the survival and growth characteristics, and nutrient transport activities, of mutant mycobacteria within mouse and human macrophages. These experiments will lead to a more complete understanding of the vacuolar environment which is the preferred ecological niche for mycobacteria within a mammalian host, and the nutrient uptake strategies employed by virulent mycobacteria to survive and grow in that intracellular environment.

Grant: 2R01AI035817-06A1
Program Director: LANG, DENNIS R
Principal Investigator: GOLDBERG, MARCIA B MD
Title: MECHANISM AND FUNCTION OF UNIPOLARITY OF SHIGELLA ICSA
Institution: MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA
Project Period: 1994/08/01-2004/11/30

DESCRIPTION (Adapted from the Applicant's Abstract): *Shigella* spp. continue to be a leading cause of dysentery and diarrhea annually worldwide. *Shigella* is unique among Gram-negative enteric pathogens in that it accesses the cytoplasm of host cells and assembles actin into long tails, which propel bacterial spread through tissues. The investigators have previously shown that the *Shigella* outer membrane protein IcsA is sufficient for actin assembly and that IcsA is localized to a single pole of the bacillus. The molecular mechanisms involved in the unipolar localization of IcsA are unknown. Their data demonstrate that IcsA is directly targeted to the bacterial pole. In conjunction with this, they have developed constructs that provide the tools necessary to directly address the molecular mechanisms of unipolar targeting of IcsA, a major goal of this proposal. In contrast, IcsA that is uniformly distributed over the surface of certain *E. coli* strains is able to mediate actin tail assembly without capping of the IcsA. This proposal also specifically addresses the function of unipolar localization of IcsA in *Shigella* pathogenesis, which they are now in a position to test critically. The Specific Aims of this proposal are: (1) the identification and characterization of residues and domains of IcsA required for its unipolar localization; (2) the identification of *Shigella* proteins that directly interact with IcsA and analysis of their potential role in IcsA unipolar localization; and (3) an assessment of the role of IcsA unipolarity in *Shigella* pathogenesis. This application proposes to obtain information that will provide insight into the molecular mechanisms of unipolar targeting of the *S. flexneri* virulence factor IcsA. Further, their studies will elucidate the role of IcsA unipolarity in the pathogenesis and virulence of *Shigella*. Finally, their studies will likely provide insight into the fundamental mechanisms that mediate three-dimensional targeting of proteins in bacteria and the molecular characteristics of the bacterial old pole that distinguish it from the new pole and the sides of the bacillus.

Grant: 2R01AI036258-05
Program Director: AULTMAN, KATHRYN S.
Principal Investigator: FALLON, ANN M
Title: MOSQUITO IMMUNITY FUNCTIONS
Institution: UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN
Project Period: 1995/07/01-2003/12/31

DESCRIPTION (Adapted from the Applicant's Abstract): This 4-year proposal describes continuing investigation of immunity functions expressed by *Aedes albopictus* and *Aedes aegypti* mosquito cell lines. In a previous funding period, it was shown that these cells express several inducible activities when stimulated with heat-killed bacteria. Activities corresponding to transferrin, defensin, lysozyme and cecropin have now been characterized at the molecular level, including recovery of corresponding cDNAs. In aim 1 they will use the cecropin and lysozyme cDNAs as probes to characterized gene family size and genomic DNA structure. The work will include identification of exon-intron organization and recovery of upstream flanking DNA, which will be analyzed for potential regulatory elements.

Grant: 2R01AI036396-06A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: RAMAKRISHNAN, LALITA PHD
Title: GENETIC BASIS OF MYCOBACTERIAL INVASION
Institution: STANFORD UNIVERSITY STANFORD, CA
Project Period: 1994/08/01-2005/01/31

Abstract Text Not Available

Grant: 2R01AI036525-05
Program Director: LANG, DENNIS R
Principal Investigator: SZTEIN, MARCELO B
Title: IMMUNE MECHANISMS OF PROTECTION IN S TYPHI VACCINES
Institution: UNIVERSITY OF MARYLAND BALT PROF SCHOOL BALTIMORE, MD
Project Period: 1994/09/01-2005/08/31

DESCRIPTION: (Adapted from Applicant's Abstract) The goal of this project is to fully characterize at the local and systemic levels T cell-mediated immune responses (CMI) induced by oral immunization with new generation attenuated *S. typhi* strains and to identify the cellular and molecular mechanisms that best correlate with protection against challenge with wild-type *S. typhi*. The development of improved typhoid vaccines has been hampered by a considerable lack of information on the specific determinants of protective CMI to *S. typhi* infection. Moreover, insufficient information is available regarding human mucosal immune responses to *S. typhi*, likely to be a key defense mechanism. Our central hypothesis is that the induction of potent and sustained CMI at both the local (e.g., the gut microenvironment) and systemic levels is critical for the development of an effective typhoid vaccine. Specifically, using peripheral blood mononuclear cells (PBMC) and mucosal biopsy specimens obtained from volunteers vaccinated with attenuated strains of *S. typhi* or challenged with wild-type *S. typhi* we propose. 1) To test the hypothesis that protective CMI responses against *S. typhi* will be determined by a set of immunodominant epitopes derived from *S. typhi* antigens. We will study the fine specificity of anti-*S. typhi* T cell responses by identifying immunodominant *S. typhi* proteins and T cell epitopes using a panel of T cell clones obtained from blood of immunized volunteers. 2) To test the hypothesis that CTL induced by immunization kills infected target cells by a combination of perforin and FAS ligand-mediated mechanisms. 3) To test the hypothesis that dendritic cells (DC) play a key role in the induction of protective immune responses in humans following oral immunization with attenuated strains of *S. typhi*. 4) To test the hypothesis that challenge with wild-type *S. typhi* or immunization with attenuated strains elicits the appearance in the gut mucosa of specific CTL effectors and T lymphocytes that produce Type-1 cytokines, and that these responses correlate with protection following challenge with wild-type *S. typhi*.

Grant: 2R01AI036929-07
Program Director: LANG, DENNIS R
Principal Investigator: THERIOT, JULIE A PHD
Title: ACTIN BASED MOTILITY OF A BACTERIAL PATHOGEN
Institution: STANFORD UNIVERSITY STANFORD, CA
Project Period: 1994/12/01-2005/02/28

Listeria monocytogenes is a ubiquitous Gram-positive bacterium that can cause serious food-borne infections in pregnant women, newborns and immunocompromised adults. The bacterium grows directly in the cytoplasm of infected host cells and moves rapidly throughout and between infected cells using a form of actin-based motility. An *L. monocytogenes* protein, ActA, induces polymerization of host cell actin to form a "comet tail" structure that pushes the bacterium through the host cell cytoplasm. The overall goal of this proposal is to understand the mechanism of the actin-based motility of *L. monocytogenes*. Three complementary approaches will be used to study this problem: biochemical, biophysical, and cell biological. A major goal is the establishment of a simplified biochemical system that can support *L. monocytogenes* motility, and development of quantitative assays so that the precise role of each component in actin-based motility can be assessed. The amount of force generated by moving bacteria will be measured directly using a laser force trap (optical tweezers) and compliant microneedles. The mechanism of coupling between actin filament polymerization and production of a motile force will be examined. Finally, videomicroscopy techniques will be used to observe the process of bacterial spread from one host cell to another, and a combination of genetic and pharmacological perturbations will be used to define the contributions of the bacterium, the host cell, and the actin-rich comet tail associated with the moving bacterium, to the process of intercellular spread. Successful completion of our research goals would give significant insight into the mechanisms by which pathogenic bacteria such as *L. monocytogenes* communicate specifically with the cells of their human hosts. This understanding might pave the way for the development of new ways to prevent and cure bacterial infections. In addition, the results of our research would contribute to our understanding of a wide variety of basic biological process involving actin-based cell movement, including wound healing, immune system responses, and embryonic development. Furthermore, since most malignant tumors do not become lethal until the cancer cells move away from the tumor site and invade other tissues, a detailed understanding of the basic mechanisms that regulate actin-based motility may also be important in the development of therapeutic strategies for combating metastatic cancers.

Grant: 2R01AI037142-05
Program Director: MILLER, MARISSA A.
Principal Investigator: CHEUNG, AMBROSE L MD MEDICINE
Title: CHARACTERIZATION OF SAR/AGR INTERACTIONS IN S AUREUS
Institution: DARTMOUTH COLLEGE HANOVER, NH
Project Period: 1996/07/01-2005/05/31

Because of increasing antibiotic resistance, *Staphylococcus aureus* continues to be a major human pathogen. To develop a novel approach against this pathogen we have tried to understand the genetic control apparatus in an attempt to identify new targets amenable to therapy. Using Tn917 mutagenesis, we identified a locus on the *S. aureus* chromosome, designated *sar*, that is involved in the regulation of several extracellular and cell wall virulence factors. The *sar* locus is composed of three overlapping transcripts, each encoding SarA, the major *sar* regulatory molecule. The SarA protein (14.5 kD) binds to the *agr* promoter region to modulate transcription of RNAII and RNAIII (the *agr* regulatory molecule) from the *agr* P2 and P3 promoters. As *agr* is a pleiotropic regulator of exoprotein synthesis, our data are consistent with the hypothesis that SarA positively regulates the expression of exoprotein genes via *agr*. The binding site of SarA on the *agr* promoter has been mapped to a 29-bp sequence in the P2-P3 interpromoter region. Sequence alignment revealed a conserved "SarA recognition motif" upstream of the -35 promoter boxes of several *sar* target genes (e.g. *hla*, *spa* and *fnbB*) that is homologous with the 29-bp sequence. Deletion of the "SarA recognition motif" in the *agr* and the *spa* promoter regions renders the respective genes unresponsive to the effect of the *sar* locus. To verify the hypothesis that SarA binds to a conserved SarA recognition motif in various target genes to modulate transcription we propose to examine the interactions of SarA with target promoters (*hla*, *fnbB* and *spa*) lacking the SarA recognition motif. These studies will be followed by footprinting and in vitro transcription assays of target promoters in the presence of SarA. These in vitro data will be confirmed by in vivo transcription study of *S. aureus* cells carrying *sar* target genes lacking the SarA recognition motif. A corollary to our hypothesis is that the activation of *sar* target genes may depend on the SarA protein level which, in turn, may be controlled by SarA and genetic elements within the extensive 800-bp *sar* promoter region. Additionally, a 13 kD protein, designated SarR, may bind to the *sar* promoter region to modulate *sar* transcription and ultimately SarA expression. We thus propose to evaluate the contribution of these genetic elements and regulatory proteins in regulating SarA expression and hence target gene transcription. The results of these studies will provide a unifying hypothesis for *sar*-mediated regulation whereby SarA binds to the conserved SarA recognition motif to control target gene transcription and that activation of these promoters is dependent on the SarA protein levels. This knowledge is indispensable if we are to design synthetic analogs to interfere with the expression of virulence genes controlled by the *sar* locus in the future.

Grant: 2R01AI037248-06A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: BARBOUR, ALAN G MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC
Title: BIOLOGY AND CONTROL OF LYME DISEASE BORRELIA
Institution: UNIVERSITY OF CALIFORNIA IRVINE IRVINE, CA
Project Period: 1994/09/30-2005/04/30

DESCRIPTION (Adapted from Applicant's Abstract): The proposed collaborative project is based collectively on investigations of the pathogenesis and epidemiology of Lyme disease, the ecology, vector biology, and population genetics of vector-borne diseases, the molecular biology of *Borrelia burgdorferi*, and vaccine development. The emphases of the proposed studies are the evolutionary biology of *B. burgdorferi* and new strategies for Lyme disease prevention. The long term goals are the following: (1) To significantly reduce the risk of Lyme disease among humans and domestic animals by vaccine intervention in the natural maintenance cycle of *B. burgdorferi* in a highly endemic area. (2) To study the evolution of *B. burgdorferi* as an infectious agent through controlled intervention studies in a highly endemic area. (3) To provide for a vaccine that is highly effective and that can be expeditiously, safely, and inexpensively administered in the field. The hypotheses for the project are the following: (A) A high prevalence of anti-OspA antibodies among *Peromyscus leucopus* and/or other mammalian reservoirs in the field will reduce the transmission rate between reservoir hosts and vector ticks, thereby reducing the prevalence of *B. burgdorferi* infection in ticks. (B) Immunization of reservoirs in the field will not alter the population structure of *B. burgdorferi* at the vaccine field sites in comparison to the control sites. (C) Transmission-blocking immunity among *P. leucopus* and/or other mammalian reservoirs can be achieved by oral as well as by parenteral immunization with a single immunogen or combined immunogens. The specific aims are the following: (1) Field studies of the effect of immunization of *P. leucopus* on the rate of transmission of *B. burgdorferi* between reservoir host and vector ticks. (2) Assessment of the effects of vaccine intervention in the field on the population structure of *B. burgdorferi* in ticks and in vertebrate reservoirs. (3) Further development of single-vaccination and orally-delivered field vaccines and evaluation of second, supplementary antigens. The proposed studies may provide insights as molecular, organismal, and population levels about *B. burgdorferi*, its transmission, and reservoir host immunize responses. The results may also have relevance for the control of other vector-borne zoonotic diseases and perhaps for development of novel methods for vaccine delivery.

Grant: 2R01AI037277-09
Program Director: BAKER, PHILLIP J.
Principal Investigator: NORRIS, STEVEN J PHD
Title: VIRULENCE-ASSOCIATED PROTEINS IN LYME DISEASE
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX
HOUSTON
Project Period: 1994/09/01-2005/06/30

DESCRIPTION (Adapted from Applicant's Abstract): Lyme disease, which is caused by infection with *Borrelia burgdorferi* and related spirochetes, is transmitted by ticks, and is characterized by dermatologic, neurologic, cardiovascular, and arthritic manifestations. *B. burgdorferi* is an invasive organism that causes disease through persistent infection and chronic stimulation of host inflammatory responses. Understanding how the organism can evade the host immune response and cause persistent infection is important for understanding its pathogenesis, and improving diagnosis, treatment, and prevention. In the previous period of the award, Dr. Norris and colleagues discovered that *B. burgdorferi* possesses an elaborate system of antigenic variation, called the VMP-like Sequence (vls) locus, because of its similarity to the Variable Major Protein system of relapsing fever *Borrelia*. A central cassette region of the vlsE gene has been identified which undergoes extensive segmental recombination with a series of vls silent cassettes located adjacent of vlsE on the linear plasmid lp28-1. VlsE protein is highly immunogenic, and immunization with it confers protection against infection with *B. burgdorferi* expressing the homologous protein, but is only partially protective against isogenic strains expressing variants of VlsE, generated by recombination in the vls locus. Additionally, a strong antibody response can be detected in animals and in humans infected with *B. burgdorferi*, indicating VlsE is immunogenic and expressed during infection. These findings indicate the discovery of a highly immunogenic protein whose ability for genetic and immunologic variation could provide the bacteria with a powerful mechanism for immune evasion. The investigator plans to build on these initial important findings to further characterize the vls system in related spirochetes that cause Lyme disease, *B. garinii*, *B. afzelii*, and other isolates of *B. burgdorferi*. The immune response to VlsE epitopes will be investigated, and the potential of a multi-valent vaccine based on variant epitopes of VlsE will be tested. The location of invariant and variant epitopes of VlsE will be determined by structural analysis, employing recombinant VlsE and making use of monoclonal antibodies to be generated against VlsE variants. The correlation of particular plasmids with infectivity will be further analyzed to help identify additional gene products important in persistence, invasion, and pathological development.

Grant: 2R01AI037606-06
Program Director: LANG, DENNIS R
Principal Investigator: DONNENBERG, MICHAEL S MD
Title: MOLECULAR BASIS OF LOCALIZED ADHERENCE IN E. COLI
Institution: UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD
SCHOOL
Project Period: 1995/04/01-2005/03/31

DESCRIPTION (Adapted from the Applicant's Abstract): Type IV pili are produced by many Gram-negative bacteria of medical and veterinary importance. The biogenesis of type IV pili appears to be quite complex, but is poorly understood. Because all of the genes required for the biogenesis of the bundle-forming pilus (BFP) type IV fimbriae of enteropathogenic E. coli (EPEC) are known, this system serves as an excellent model to dissect this process. The central hypothesis of this proposal states that BFP are assembled by a multi-component Bfp machine that is made up of the products of the genes of the bfp operon. The investigators plan to further their studies of BFP in an effort to progress toward a complete understanding of the type IV pilus assembly machine. Because of the complexity of the type IV pilus biogenesis machinery, they find it convenient to conceptualize the whole as consisting of two subassemblies, which they intend to define. To those ends, they propose four specific aims. First, they will determine whether each Bfp protein is located in the cytoplasm, periplasm, inner or outer membrane. This information is needed to refine hypothesis regarding which Bfp proteins directly interact. Second, they will describe the architecture of the Bfp outer membrane subassembly. Evidence from their lab and other labs suggests that BfpB, BfpG and BfpU interact with each other in the outer membrane. Since they have already developed a functional, purified histidine-tagged BfpU fusion protein, they will use BfpU as a tool to define the interactions among these three proteins. Third, they will describe the architecture of the Bfp inner membrane assembly. They have examined the topology of the BfpE protein and found that it contains three transmembrane domains that span the inner membrane. They will use this knowledge as a basis for investigating interactions among BfpE, BfpC, BfpD and BfpF. Fourth, they will identify the proteins that link the inner and outer membrane subassemblies of the Bfp machine. One hypothesis states that BfpI, BfpJ, BfpK and BfpL serve this function. As data are gathered from specific aims 1-3 they will modify and test this hypothesis. From the results of these studies a clearer picture of the overall design of the type IV pilus biogenesis machine will emerge. Further studies including determining binding constants and atomic structure will be required to reveal how the machine functions to assemble these important virulence factors.

Grant: 2R01AI037720-05A1
Program Director: LANG, DENNIS R
Principal Investigator: ZYCHLINSKY, ARTURO PHD
Title: APOPTOSIS IN SHIGELLA INFECTIONS
Institution: NEW YORK UNIVERSITY SCHOOL OF MEDICINE NEW YORK, NY
Project Period: 1996/03/01-2005/05/31

Shigellae are the etiological agents of bacillary dysentery, a severe form of diarrhea that is often fatal in infants. Shigellosis is an acute inflammatory disease. Here we propose to investigate the role of apoptosis in the initiation of inflammation. We have demonstrated that Shigella induces apoptosis in macrophages in vitro and in vivo. Shigella first invades cells and then escapes from the phagocytic vacuole into the cytoplasm. In the cytoplasm, Shigella secretes the plasmid-encoded virulence factor Invasion Plasmid Antigen (Ipa) B which is necessary to induce cell death. IpaB binds to caspase (Casp)-1, a host cysteine protease that is required for Shigella induced apoptosis. Apoptosis mediated by Casp-1 appears to be pro-inflammatory in Shigella infections, since Casp-1 proteolytically activates the cytokines pro-Interleukin (IL)-1beta and pro-IL-18. Macrophages infected with Shigella release mature IL-1beta and IL-18. Furthermore, casp-1 knock-out mice do not mount an acute inflammation in response to Shigella infection. In vivo, some apoptotic cells are localized to regions of the lymphoid follicle where Shigella is not detectable. This difference in distribution suggested that Shigella possesses a second cytotoxic molecule, not IpaB, that can diffuse within infected tissue. We identified the novel diffusible cytotoxic activity in Shigella culture supernatants as Bacterial Lipoproteins (BLP). We also demonstrated that BLP activates both apoptosis and the host cell transcription factor Nuclear Factor - kappa B (NF-kappaB) through the Toll Like Receptor (TLR)2. In this application we propose to further understand the significance of apoptosis in Shigella infections. More specifically we will determine: (1) the role of Casp-1 activated cytokines in acute inflammation and whether apoptosis is required for the release of mature IL-1beta and IL-18 and (2) the signal transduction pathway activated by TLR2 after treatment with BLP and the role of BLP and TLR2 in vivo.

Grant: 2R01AI037750-05

Program Director: LANG, DENNIS R

Principal Investigator: FOX, JAMES G DVM VET
MEDICINE:VETERINARY
MEDICINE-UNSPEC

Title: IN VIVO PATHOGENESIS OF HELICOBACTER PYLORI

Institution: MASSACHUSETTS INSTITUTE OF CAMBRIDGE, MA
TECHNOLOGY

Project Period: 1996/09/01-2005/08/31

DESCRIPTION (Adapted from the Applicant's Abstract): *H. pylori*, an infection approaching 100 percent in developing countries, has been strongly linked epidemiologically to gastric cancer, but the mechanism and cofactors required for gastric cancer are poorly understood. Furthermore, it is not known at what stage in progression to gastric cancer that eradication of *H. pylori* would interrupt the carcinogenic process. Polyparasitism is also ubiquitous in developing populations where *H. pylori* is endemic. The investigators have developed a C57BL/6 mouse model of chronic *H. pylori/felis* gastritis that is characterized by the progressive development of gastric atrophy, intestinal metaplasia and invasive gastric cancer. The mechanism of lesion development appears to involve increased apoptosis, mucus neck proliferation, intestinal metaplasia leading to altered cellular differentiation and changes in mucin phenotype and progression of invasive cancer in submucosal vasculature. They have also investigated bacterial and environmental factors that influence disease pathogenesis by generating isogenic mutants lacking specific candidate virulence determinants and by maintaining *Helicobacter* infected animals on diets high in salt. They have recently shown that in mice coinfecting with *Helicobacter* and a helminth infection, *H. polygyrus*, the gastric cytokine Th1/Th2 profile switches and the gastric phenotype changes from a Th1 to a Th2 type gastritis. They now propose to explore the effects of specific genetic alterations, environmental influences and coinfections on the mucosal response and progression of *Helicobacter* associated gastric lesions. Specifically, they will ask whether 1) progression of *H. pylori* gastritis can be interrupted at critical points in the disease by antimicrobials or therapeutic vaccination to prevent development of premalignant lesions and gastric adenocarcinoma in the gerbil and/or mouse model 2) Alternatively, do environmental factors such as dietary salt, accelerate or otherwise alter the carcinogenic process, and importantly does the strain of *H. pylori* (with and without specified pathogenic determinants) influence the outcome of gastric disease in the mouse and gerbil model 3) Does modulation of the Th1/Th2 axis of the immune system by various helminth infections influence the severity and progression of gastritis in rodent models. Overall, these rodent models of *Helicobacter* infection will be used to study the mechanism by which *Helicobacter* contributes to neoplasia, and the factors (host, bacteria, dietary or co-infections) which confer susceptibility and/or resistance to premalignant lesions and gastric cancer.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2R01AI038273-06
Program Director: RUBIN, FRAN A.
Principal Investigator: CAPARON, MICHAEL G PHD MICROBIOLOGY, OTS
Title: VIRULENCE, OXYGEN AND PROTEIN F IN STEPTOCOCCUS PYOGENES
Institution: WASHINGTON UNIVERSITY ST. LOUIS, MO
Project Period: 1995/06/01-2005/05/31

We currently have a very poor understanding of why some infections by the Gram positive pathogen *Streptococcus pyogenes* result in mild self-limiting disease, while others progress to life-threatening tissue destructive diseases. To a large extent, this reflects our poor understanding of the critical events that occur during the initial stages of the interaction of the organism with human tissue. The long term goal of this project has been to characterize these events in molecular detail and to develop techniques to facilitate this analysis. A central observation has been that expression of a major fibronectin binding adhesin, protein F, is linked to pathways which sense oxidative stress. This suggests that *S. pyogenes* must adapt to the effects of toxic oxygen chemistry during the initial stages of infection and that sensing oxidative stress provides an important signal for expression of virulence. The testing of this hypothesis will be the focus of this proposal. Three specific aspects of this question will be addressed. Previous work has shown that prtF is regulated by several distinct pathways, one of which involves the transcriptional activator RofA. Both RofA-dependent and -independent pathways have a common and absolute requirement for a specific site in the prtF promoter that is bound by RofA. This suggests that other RofA-like proteins (RALPs) present in the genome may regulate prtF. Testing this will involve characterization of the DNA-recognition domain of RofA, and the contribution of RofA and RALPs to virulence and regulation of other genes whose promoters contain RofA-binding sequences. The second aspect will be directed at further characterization of the Gas locus, a two-component regulator of unusual structure. While Gas mutants grow normally under anaerobic conditions, they lose their ability to grow under aerobic conditions. Identification of the regulatory targets of this locus may reveal novel genes required for resistance to oxidative stress. The third component of the project will involve characterization of an induced resistance response to peroxide stress that involves no previously known peroxidase or regulator. Finally, the ability of these mutants to cause disease in a murine model of cutaneous disease will be evaluated. This will provide a direct test of the hypothesis that genes which are required for resistance to oxidative stress and growth in an aerobic environment will be important for survival in inflamed tissue. Since most of these genes are unique, a number of promising candidate drug targets may be identified.

Grant: 2R01AI038446-05
Program Director: KLEIN, DAVID L
Principal Investigator: WEISER, JEFFREY N
Title: ROLE OF OPACITY VARIATION IN PNEUMOCOCCAL PATHOGENESIS
Institution: CHILDREN'S HOSPITAL OF PHILADELPHIA PHILADELPHIA, PA
Project Period: 1996/04/01-2000/11/30

DESCRIPTION (Adapted from the Applicant's Abstract): This is a continuation of a grant that has concentrated on *Streptococcus pneumoniae* and its ability to adapt to the nasopharynx with colonization as well as to produce invasive infection with bacteremia. Such adaptation has been shown to be correlated with the expression of two distinct phenotypes, the opaque and transparent colony forms. Opacity phenotype is associated with on-off switching of pyruvate oxidase (SpxB), that mediates the aerobic metabolism of pyruvate and results in the generation of H₂O₂. Pursuing the biochemical and genetic basis of opacity variation will continue with a focus on the ability of the pneumococcus to produce and tolerate unusually high levels of H₂O₂. The overall goal of the proposal is to define the effect of opacity phenotype on carriage, the important first step in the pathogenesis of pneumococcal disease. The specific aims are: (1) to define the contribution of H₂O₂ production and opacity variation to pneumococcal carriage; (2) to characterize the physiology of H₂O₂ production; (3) to define the mechanism for resistance to high-level H₂O₂ production. A genetic approach here will be used to attempt to isolate sequences contributing to the resistance to endogenously generated peroxide; and (4) to identify and characterize regulatory elements controlling opacity (phenotypic) variation. The hope here is to isolate transcription factors that may function as global regulators affecting pyruvate oxidase.

Grant: 2R01AI038947-05
Program Director: LAUGHON, BARBARA E.
Principal Investigator: KHOSLA, CHAITAN S
Title: RIFAMYCIN BIOSYNTHETIC ANALOGS
Institution: STANFORD UNIVERSITY STANFORD, CA
Project Period: 2000/09/15-2004/08/31

DESCRIPTION: (Principal Investigator's Abstract) The long term goal of this project continues to be the production of analogs of rifamycin and evaluation of their activity as antitubercular drugs. We hope to develop forms with activity against Mycobacterium sp. that have become resistant to rifampicin and(or) with improved potency over this drug and the three other rifamycin analogs in current use clinically. Our working hypothesis is based primarily on elimination of functionalities that are modified by the three types of resistance mechanisms used by M. tuberculosis, but we also envisage the possibility of altering the drug's binding to the RNA polymerase beta-subunit, which is the most prevalent resistance mechanism in other bacteria. The analogs will be prepared by combinatorial biosynthesis, using modified polyketide synthase genes. The following specific aims will be pursued in the Hutchinson and Khosla laboratories at Wisconsin and Stanford, respectively, in collaboration with the Floss laboratory at Washington. (1) Develop a collection of *Amycolatopsis mediterranei* strains with deletions in different PKS genes suitable for expression of individual PKS genes and reconstitution of active PKS in vivo. (2) Establish the minimum number of genes required to form proansamycin X or rifamycin W in *A. mediterranei* and in a heterologous host such as *Streptomyces lividans*. (3) Explore the behavior of the Rif PKS in vivo and in vitro, in an attempt to understand why the intermediates of polyketide chain assembly are spontaneously released from the enzyme subunits at a high frequency. (4) Systematically modify the rif PKS genes by domain and module replacement targeted at altering position 7 of the naphthoquinone ring and positions 21 to 25 in the ansamycin ring. (5) Engineer the rifamycin gene cluster for production of polyketides related to geldanamycin through the use of hybrid PKSs made from rif and ery genes. (6) Engineer the rifamycin gene cluster for overproduction of rifamycin B and eventual rifamycin analogs. (7) If necessary, move some of the rifamycin gene cluster into *Streptomyces lividans* to facilitate aims 4 - 6.

Grant: 2R01AI038991-05
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: MORRISON, RICHARD P
Title: IMMUNITY TO CHLAMYDIAL GENITAL INFECTION
Institution: MONTANA STATE UNIVERSITY (BOZEMAN) BOZEMAN, MT
Project Period: 1996/06/01-2005/08/31

DESCRIPTION (Adapted from the Applicant's Abstract): Chlamydia trachomatis is possibly the most common sexually transmitted bacterial pathogen in the world. In the United States, 4 million new cases of C. trachomatis urogenital tract infection occur each year, and it is estimated that the cost of treating those infections approaches \$4 billion annually. Urogenital infections caused by C. trachomatis result in a number of diverse clinical conditions. Infections in women range from acute self-limiting infections to more serious infections that result in pelvic inflammatory disease, infertility and ectopic pregnancy. Considerable progress has been made in the past few years to significantly broaden our understanding of immune responses that develop during the course of chlamydial infection. However, our understanding of effector mechanisms that limit chlamydial infection and prevent reinfection is insufficient. The investigator's recent data suggest that both CD4+ T cells and B cells (antibody) contribute to adaptive immunity to chlamydial genital tract infection. Thus the overall goal of this project is to use the murine model of C. trachomatis genital tract infection to study the relationship between CD4+ T cells and antibody in adaptive immunity to infection. That goal will be realized through the studies described in 4 specific aims: 1) To determine the ability of immune B cells and antibody to reconstitute protective immunity in CD4-depleted B cell deficient mice; 2) To determine if the lack of mature B cells in B cell gene knockout mice affects the development of chlamydial-specific memory T cell responses; 3) To determine the effect of simultaneous immune cell depletions on acquired immunity; and 4) To evaluate the inhibitory effects of antibodies and lymphocytes on chlamydial growth in vitro (antibody dependent cellular cytotoxicity). These studies will broaden our understanding of how the host resists chlamydial infection, and may provide new insights into the formulation and administration of an effective vaccine to control the spread of chlamydial infections or prevent the serious sequelae of disease pathogenesis.

Grant: 2R01AI040297-04
Program Director: HALL, B. FENTON
Principal Investigator: LEVINE, MYRON M MD OTHER CL
MED:CLINICAL
MEDICINE,UNSPEC
Title: BACTERIAL LIVE VECTOR-BASED VACCINE AGAINST MALARIA
Institution: UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD
SCHOOL
Project Period: 1997/04/01-2005/06/30

DESCRIPTION: (Adapted from Applicant's Abstract) The underlying hypothesis of this research plan is that it will be possible, by appropriate manipulation of attenuated *Salmonella typhi* and *Shigella* live vectors, to develop a mucosally administered multivalent vaccine to prevent *Plasmodium falciparum* malaria. Proving the broad hypothesis requires that we adapt an attenuated *Shigella* vaccine strain to deliver eukaryotic expression plasmid-based "naked" DNA (hereafter referred to as DNA vaccine) via mucosal immunization, thereby priming the immune system to recognize *P. falciparum* antigens. We will then attempt to boost the immune response by administering via mucosal immunization attenuated *S. typhi* live vectors expressing protective antigens from the sporozoite, liver, and asexual erythrocytic stages of *P. falciparum* life cycle. When using such a "prime/boost" strategy in animals by priming with parenteral DNA vaccine and then boosting parenterally with a live vector expressing the relevant antigen, the immune responses and the level of protection elicited markedly exceeds that achieved when either DNA or live vector is used for both prime and boost, or when protein is used to boost following priming with DNA. The efficacy of DNA prime/live vector boost has been particularly impressive with *Plasmodium* antigens. To optimize the priming potential of *Shigella* live vectors harboring DNA vaccines, we propose to use DNA vaccines in which codon usage has been optimized for expression by mammalian (human) cells. We also propose to optimize the boosting potential of *S. typhi* live vectors carrying prokaryotic expression plasmids at two independent levels: 1] modifying the codon usage within *falciparum* open reading frames for optimum expression within *S. typhi* live vectors; and 2] exploiting a recently developed *S. typhi* plasmid maintenance system which employs partition and post-segregational killing functions, to maintain codon-optimized expression plasmids. Since we propose to study *Plasmodium* genes encoding CSP, LSA-1, SSP2, and MSP-1, we will examine the equivalence of priming with a mixture of four *Shigella* live vector strains, each carrying a plasmid encoding a distinct *P. falciparum* antigen, versus immunization with each individual live vector; we will also examine the equivalence of boosting with *S. typhi* either as a combination vaccine or as separate constructs. We hypothesize that by independently attaining partial protection against further development of each of the stages of *P. falciparum*, and by these effects working in concert, we will ultimately be able to prevent disease completely in some subjects and to markedly ameliorate disease severity in others.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01AI042827-01A2
Program Director: RUBIN, FRAN A.
Principal Investigator: PANCHOLI, VIJAYKUMAR PHD
Title: ROLE OF SURFACE DEHYDROGENASE IN STREPTOCOCCAL INFECTION
Institution: ROCKEFELLER UNIVERSITY NEW YORK, NY
Project Period: 2000/05/01-2001/04/30

The PI hypothesizes that a surface dehydrogenase (SDH) plays a critical role in the early events of infection by facilitating entry of group A streptococcus into pharyngeal cells. The author states that SDH is essential for survival of GAS and that SDH has multiple means of binding to several mammalian proteins and also has ADP-ribosylating activity. SDH interacts with mammalian pharyngeal cells and regulates the signal transduction pathways in pharyngeal cells which facilitates adherence and penetration of the host cell. Specific Aim 1. A 32 kDa pharyngeal cell membrane receptor for SDH will be purified and biochemically characterized Specific Aim 2. The receptor binding domain of SDH will be identified using several protein and site directed mutagenesis techniques to establish the molecular basis of SDH-pharyngeal cell receptor interaction. Specific Aim 3 Using intact streptococci and recombinant native and mutated SDH proteins, SDH mediated signaling pathways in pharyngeal cells will be elucidated by studying various intracellular signaling events. Specific Aim 4, native and mutated SDH will be covalently linked to fluorescinated microbeads to study adherence and invasion of cell lines.

Grant: 1R01AI043389-01A2
Program Director: LANG, DENNIS R
Principal Investigator: BLISKA, JAMES B PHD
Title: MODULATION OF HOST SIGNALING FUNCTIONS BY YERSINIA YOPS
Institution: STATE UNIVERSITY NEW YORK STONY BROOK STONY BROOK, NY
Project Period: 2000/02/01-2005/01/31

DESCRIPTION (Adapted from the Applicant's Abstract): The human-pathogenic *Yersinia* spp. (*Y. pestis*, *Y. enterocolitica*, and *Y. pseudotuberculosis*) are responsible for a range of diseases including diarrhea, mesenteric lymphadenitis, and bubonic plague. These bacteria invade into and colonize the lymphatic organs of humans and a variety of animal hosts. Colonization of a host by *Yersinia* requires the function of a plasmid-encoded contact-dependent type III secretion system. This type III system translocates a set of toxic proteins known as Yops into host cells. The Yops impair normal host cell signaling functions, resulting in inhibition of phagocytosis, suppression of cytokine synthesis, and induction of apoptosis. The long-term goal of this grant is to understand how Yops modulate host cell signaling functions. The investigators will focus their studies primarily on YopH, a protein tyrosine phosphatase that inhibits phagocytosis, and YopJ, a protein that prevents cytokine synthesis and induces apoptosis. The first specific aim is to carry out a structure/function analysis of an amino-terminal domain in YopH that mediates translocation and substrate recognition. A combination of biophysical and genetic approaches will be used to achieve this goal. The second specific aim is to examine the mechanism of substrate recognition by YopH inside host cells. Animal and cultured cell infection assays will be used to study the behavior of genetically-altered YopH proteins *in vivo*. The third specific aim is to analyze the interaction of YopJ with host target proteins and to elucidate its mechanism action. Mutant forms of YopJ unable to bind target proteins will be generated and analyzed for biological activity in animal and cultured cell infection assays. The possibility that other Yops modulate the activities of mitogen-activated protein kinases in host cells will also be explored. As type III secretion pathways are important virulence determinants in a large number of bacterial pathogens, and the Yops provide an extremely powerful system to study pathogen interference with host signaling functions, these studies will aid the development of new strategies to combat a variety infectious diseases.

Grant: 1R01AI043412-01A2
Program Director: MILLER, MARISSA A.
Principal Investigator: AXELSEN, PAUL H MD
Title: STRUCTURE-BASED ANTIMICROBIAL DESIGN
Institution: UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA
Project Period: 2000/04/01-2005/03/31

DESCRIPTION (adapted from applicant's abstract): This is a proposal to apply the techniques of X-ray crystallography, molecular dynamics simulation, and infrared spectroscopy, with organic synthesis, in a coordinated interdisciplinary effort to better understand ligand recognition by glycopeptide antibiotics, and develop new agents effective against vancomycin-resistant bacteria. We aim to determine the structure of ligand complexes with glycopeptide antibiotics using X-ray crystallography, experimentally verify aspects of ligand recognition behavior predicted by computer simulation, and synthetically alter the natural specificity of glycopeptide antibiotic in a way which enhances its affinity for cell wall fragments of vancomycin-resistant bacteria. We emphasize the use of molecular dynamics computer simulations for interpreting results and guiding experimental strategies. The overall aim is to confront the emerging health threat of vancomycin-resistance by facilitating the rational design of drugs, and gain insight into the physico-chemical basis of specific molecular recognition.

Grant: 1R01AI043428-01A2
Program Director: MILLER, MARISSA A.
Principal Investigator: ROSEN, BARRY P
Title: METAL BINDING DOMAINS IN METALLOREGULATORY PROTEINS
Institution: WAYNE STATE UNIVERSITY DETROIT, MI
Project Period: 2000/08/01-2004/07/31

The goal of this project is determination of the structure of the metal binding sites that form the inducer binding domains of transcriptional repressors that regulate plasmid-encoded bacterial resistances. In common with many drug and antibiotic resistances, the arsenical (ars) and cadmium (cad) resistance operons encode transport ATPases for the extrusion of As(III)/Sb(III) or Pb(II)/Cd(II)/Zn(II). The ArsR and CadC repressors are two small homologous metalloregulatory proteins responsible for metal-regulated gene expression of the ars and cad operons. Residues required for each inducer binding domain will be determined. In addition, in ars operons there is a second As(III)/Sb(III)-responsive repressor, ArsD, which does not exhibit homology to any known metal binding protein. ArsR and ArsD form a regulatory circuit that senses both low and high concentrations of environmental metalloids. The residues involved in As(III)/Sb(III) binding to ArsD will be determined. Finally, the copper (copA) resistance gene encodes a Cu(I)-translocating P-type ATPase. Expression of copA is regulated by copper or silver. The putative CopR regulatory protein will be identified and characterized. Our studies will define new classes of metal binding motifs. This may have more general applicability, since nearly all transport-related drug resistances are transcriptionally regulated, and the drug binding motifs of the regulatory proteins are largely unknown. Thus the repressors of the genes for these efflux pumps provide good model systems for the study of regulation of transmissible bacterial antibiotic resistances.

Grant: 1R01AI043528-01A2
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: CAMPOS-NETO, ANTONIO
Title: VACCINE DEVELOPMENT IN TUBERCULOSIS
Institution: INFECTIOUS DISEASE RESEARCH INSTITUTE SEATTLE, WA
Project Period: 2000/09/01-2005/08/31

This proposal is designed to identify, characterize, and clone *Mycobacterium tuberculosis* genes encoding proteins associated with specific T cell responses of presumed protected humans and animals. Antigens associated with both CD4+ and/or CD8+ T cell responses are the target of this proposal. The recent identification, by our group, of a novel and protective antigen opens new possibilities for vaccine development. Two other antigens/gene have been cloned, expressed, and purified. They will be evaluated for their ability to elicit T cell responses from human PBMC, primarily from non-BCG immunized PPD+ healthy individuals. The recombinant antigens alone and in combination will also be evaluated as vaccine candidates in three animal protection models, including mice, guinea pigs, and cynomolgus monkeys, all of which will be challenged by aerosol or intratracheal routes. In addition to the evaluation of these antigens, we will characterize and clone other vaccine candidates using recently developed approaches for the direct identification of both CD4 and CD8 T cell antigens.

Grant: 1R01AI043617-01A2
Program Director: LANG, DENNIS R
Principal Investigator: ALLEN, LEE-ANN H PHD
Title: INTERACTION OF HELICOBACTER PYLORI WITH PHAGOCYTES
Institution: UNIVERSITY OF IOWA IOWA CITY, IA
Project Period: 2000/09/29-2004/08/31

Description (Adapted from the applicant's abstract): *Helicobacter pylori* is a gram negative bacterium which colonizes the gastric epithelium of up to half of the world's population and plays a causative role in the development of gastric and duodenal ulcers and gastric adenocarcinoma. One of the hallmarks of *H. pylori* is its persistence, and bacteria are not cleared by the host immune system. This may be explained in part by the fact that *H. pylori* is readily phagocytosed by macrophages, but the internalized bacteria are not killed. Significantly, preliminary data obtained by the PI suggest the following hypothesis; *H. Pylori* survives for at least 20 hours inside macrophages by disrupting phagosome maturation. Moreover, this appears to occur by a novel mechanism that involves 1) delayed phagocytosis 2) homotypic fusion of early phagosomes and 3) bacteria-stimulated secretion of lysosomal enzymes from infected cells. The long-term objective of this study is to dissect the mechanism of *H. pylori* survival in macrophages at the molecular level and to identify the host and bacterial factors required for this process. Specifically, the PI will characterize the *H. pylori* phagosome in macrophages and use immunofluorescence and confocal microscopy to quantify phagosome pH; electron microscopy to determine phagosome structure; and video imaging of live cells to determine whether *H. pylori* phagosomes interact with the endosomal compartment. Subcellular fractionation and Western blotting, and immuno-electron microscopy, and antisense oligonucleotides will be used to define the roles of phosphatidylinositol 3-kinase, protein kinase C-zeta, and rab5 in phagocytosis of *H. pylori*. In addition, whether macrophage-activating cytokines and/or serum opsonins increase phagocytic killing of *H. pylori* will be determined. Finally, *H. pylori* mutants with known mutations in urease and VacA will be used to assess whether these bacterial factors are essential for bacterial survival inside macrophages. These data may be the first indication that *H. pylori* can disrupt phagosome maturation in macrophages. A complete dissection of this process at the molecular level may lead to novel therapies for treatment of *H. pylori* infection and reduce the significant morbidity associated with ulcer disease.

Grant: 1R01AI043653-01A2
Program Director: KLEIN, DAVID L
Principal Investigator: MCDANIEL, LARRY S
Title: GENETIC IMMUNIZATION AGAINST EXTRACELLULAR BACTERIA
Institution: UNIVERSITY OF MISSISSIPPI MEDICAL JACKSON, MS
CENTER
Project Period: 2000/04/01-2005/03/31

Description (Adapted from the applicant's abstract): PspA, a surface protein present on all pneumococci, acts as a virulence factor that can elicit protective antibodies in mice. Surface-attached PspA inhibits the clearance of pneumococci from the blood of mice, probably by inhibiting opsonophagocytosis, although the mechanism of action is not known. The genes encoding PspA from several different strains of the pneumococcus have been sequenced; the protein has a conserved choline-binding domain (involved in the attachment of PspA to the pneumococcal surface) and a variable α -helical coiled-coil domain with epitopes that elicit protective antibodies. A proline-rich domain between the other two domains may allow the protein to span the bacterial cell wall. Immunization of mice with a plasmid containing *pspA/Rx1* provided protection against otherwise fatal pneumococcal challenge (one of the first reports of successful genetic immunization against an extracellular bacterial pathogen. The proposed studies will identify those fragments of *pspA* that are expressed most efficiently in eukaryotic cells. A genetic construct expressing the entire α -helical domain has already been tested and it elicits protection against pneumococcal challenge. Additional constructs from within the α -helical domain will be made and tested to identify the optimal fragment for use in genetic immunization. Cytokines will also be used to direct the immune system to a Th2 antibody-mediated response. These studies should provide information on those cytokines important in eliciting protective immunity against extracellular bacterial pathogens during genetic immunization, information that might prove more generally useful than just to pneumococcal immunization. The PI will also examine the level of cross protection elicited by genetic immunization with *pspA*.

Grant: 1R01AI043965-01A2
Program Director: MILLER, MARISSA A.
Principal Investigator: ABU KWAIK, YOUSEF PHD
Title: Receptor-Mediated Endocytosis of Legionella Pneumophila
Institution: UNIVERSITY OF KENTUCKY LEXINGTON, KY
Project Period: 2000/07/15-2005/06/30

Description (Adapted from applicant's abstract): The ability of Legionella pneumophila (Lpn) to cause pneumonia is dependent on intracellular replication within pulmonary phagocytic and epithelial cells. In the environment, the bacteria are ubiquitous where they multiply intracellularly with amoebae. Invasion and intracellular replication within protozoa play major factors in the amplification and dissemination of Lpn in the environment and in transmission and infectivity to humans. We have recently shown that uptake of Lpn by the protozoan Hartmannella vermiformis is mediated by bacterial attachment to a b2 integrin-like Galactose/N-acetyl-galactosamine lectin on the protozoan surface. Bacterial attachment to the lectin is associated with induction of protozoan gene expression and with tyrosine dephosphorylation of the lectin and several cytoskeletal proteins including actin, focal adhesion kinase, paxillin, and vinculin. Our data suggested a cytoskeletal disruption in the protozoan host upon bacterial attachment. Transmission electron microscopy showed that these bacterial-induced manipulations of cell processes in the protozoan host are associated with entry of the majority of the bacteria through a cup shape-like invagination that resemble receptor-mediated endocytosis, but some bacteria are internalized by coiling phagocytosis. Our preliminary data suggest that the mechanism of entry of Lpn is novel, which may contribute to its subsequent intracellular fate. Our hypothesis is that the lectin is a protozoan receptor involved in uptake of Lpn, and is dissociated from the cytoskeleton upon bacterial attachment and invasion. Our specific aims are, 1) to clone the lectin encoding gene and examine its regulation of expression; 2) to clone and characterize the bacterial ligand that binds the lectin receptor and the mode of ligand-receptor interaction; 3) to determine cellular distribution of the lectin receptor and its subsequent fate after internalization; and 4) to evaluate the interaction of the receptor with the cytoskeleton. The results derived for the proposed studies will uncover new paradigms of uptake of intracellular pathogens and will contribute to our understanding of targeting of molecules into a "protected vacuole" inside eukaryotic cells. Our proposed studies may also facilitate the design of future preventive strategies to control the amplification and spread of Lpn in the aquatic environment, which is the only source of bacterial transmission to humans. Our studies may uncover potential pathogenic evolution of Lpn to invade the more evolved mammalian cells, and may contribute to the understanding of invasion of protozoa by Mycobacterium and Chlamydia.

Grant: 1R01AI044002-01A2
Program Director: KLEIN, DAVID L
Principal Investigator: SMITH, ARNOLD L MD PEDIATRICS:INFECTIO
DISEASES
Title: INVASIVE NONCAPSULATED H. INFLUENZAE
Institution: UNIVERSITY OF MISSOURI COLUMBIA COLUMBIA, MO
Project Period: 2000/04/01-2005/03/31

Description (Adapted from the applicant's abstract): A small percentage of invasive H. influenzae disease is (and probably has been) due to nontypable (i.e. unencapsulated) strains. With increasing immunization of children with H. influenzae conjugate capsular vaccines, the relative proportion of invasive disease due to nontypable organisms has increased: in certain locales it is the majority, in others only its prevalence has increased. Because of the concern that such cases will continue to increase in prevalence (because of the selective pressure exerted by vaccination against type b strains), it is important to understand the mechanism(s) used by unencapsulated H. influenzae to invade and cause bacteremia (and bacteremia-associated diseases) in immunocompetent immunized children. Our preliminary data indicate that the virulence determinant is carried by a novel bacteriophage (HP2) and the bacterium to replicate in blood by evading the complement system. Complement is recognized to be important in host defense against invasive H. influenzae infections in humans. The gene responsible for virulence has been designated the Human Serum Resistance 1 (hsr1) gene, and has no homolog in the current databases. Lysogenic conversion of only certain nontypeable H. influenzae results in conversion to virulence suggesting that "accessory" genes are necessary, for full expression of virulence. The proposed research seeks to define the additional gene(s) responsible for the unusual virulence properly, determine the prevalence of these virulence genes in a panel on invasive nontypeable H. influenzae, define the mechanisms by which HP2 mitigates complement activity, and identify the accessory genes needed for expression of this virulence trait using the avirulent laboratory strain H. influenzae Rd. KW20. Understanding this virulence mechanism, which is unusual for unencapsulated H. influenzae, will permit identification of new surface components, which are potential vaccinogens. Antigenic epitopes of the gene products operative in this mechanism can be used to produce a new H. influenzae conjugate vaccine active against invasive isolates.

Grant: 1R01AI044101-01A2
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: WIENER-KRONISH, JEANINE P MD
MULTIDISCIPLI:MULTIDIS
LIN, BASIC MED
Title: BIOLOGY OF PCR V
Institution: UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA
Project Period: 2000/05/01-2005/04/30

Nosocomial pneumonia is the second most common nosocomial infection and the leading cause of death from infection acquired in the hospital. *P. aeruginosa* is the most frequent gram negative bacteria involved in nosocomial pneumonia, and nosocomial pneumonias associated with *P. aeruginosa* infections have up to a 60% mortality despite appropriate antibiotic treatment. Also patients who are chronically infected with *P. aeruginosa* (i.e.: cystic fibrosis, HIV patients and bronchiectasis patients) become resistant to antibiotics and may die from their infections. Thus, there is an urgent need for novel treatments of *P. aeruginosa* infections. The long-term objectives of this grant are to determine the cell biology of a Pseudomonal protein, PcrV. PcrV is part of the bacterial type III secretory system; PcrV is involved in the translocation of bacterial toxins by *P. aeruginosa* into eukaryotic cells. It is also highly homologous to LcrV, a *Yersinia* protein also involved in the translocation of that bacteria's toxins into eukaryotic cells. Antibodies to LcrV can protect animals from infections caused by *Y. pestis* and other *Yersinia* strains. Yet, although there are similarities between LcrV and PcrV, there are also important differences in the roles of LcrV compared to PcrV in the regulation of toxin secretion in the two strains. Therefore, PcrV warrants independent investigation. This group has shown that PcrV is accessible to antibody neutralization, that antibody attachment to PcrV blocks the translocation of the Pseudomonal toxins into eukaryotic cells and that antibody to PcrV protects animals infected with virulent *P. aeruginosa* from lung injury, sepsis and death. Therefore, therapies targeting PcrV appears clinically useful. Finally, many virulent gram negative bacteria utilize the type III secretory system which delivers bacterial toxins into eukaryotic cells. These gram negative bacteria, including enteropathic *E. coli*, *Yersinia*, *Salmonella*, produce bacterial proteins and structures similar to those found in *P. aeruginosa*. Therefore, understanding the mechanism of PcrV's role in bacterial translocation into eukaryotic cells may help in the development of other therapies targeting this widespread gram negative bacterial secretory system.

Grant: 1R01AI044102-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: DUMLER, JOHN S MD MEDICINE
Title: EHRLICHIA-GRANULOCYTE INTERACTIONS AND INFECTION
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 2000/09/01-2004/08/31

DESCRIPTION (Adapted from the Applicant's Abstract): Human Granulocytic Ehrlichiosis (HGE) is a recently emergent tick-transmitted infectious disease that has been reported with increasing frequency during the last decade. The causative agent is classified within the ehrlichial genogroup II and is considered to be a variant or subspecies of Ehrlichia phagocytophila. Although many and perhaps most of the human infections are either subclinical or resolve without diagnosis, severe disease does result in prolonged hospitalization with intensive care and can progress to cause death. The manifestations of human disease are not specific but may manifest as a toxic shock-like illness, adult respiratory distress syndrome (ARDS), and fatal cases have an association with opportunistic infections. A similar association has been reported with the domestic animals infected by E. phagocytophila group organisms. The natural target cell is primarily neutrophils, although premyelocytic cells can be infected in vitro and myeloid progenitors have been hypothesized to be a site of infection in vivo. In contrast, infection of macrophages and monocyte-like cell lines is abortive and the organisms are killed. Interestingly, the severity of disease in humans does not reflect a high circulating ehrlichial load. Previous data from animals infected with E. phagocytophila indicates impaired neutrophil function - including chemotaxis, phagocytosis, and killing. These "deactivation" phenotypes may be concomitantly associated with "activation" in which binding to endothelial cells occurs and culminates in release of proinflammatory cytokines by endothelial cells and especially macrophages, leading to the triggering of a toxic-shock like injury. The PI proposes the following hypotheses: 1) E. phagocytophila group ehrlichiae bind to CD15-associated granulocyte surface receptors and initiate endocytosis via their major surface protein (MSP) antigens; 2) Binding, internalization, and propagation of E. phagocytophila group ehrlichiae initiate cytokine/chemokine expression and changes in granulocyte activation/inactivation and function; and 3) E. phagocytophila group ehrlichiae potentially influence host cell function by expressing proteins that directly interact with DNA regulatory components in the host cell's chromosomes. These hypotheses will be tested using five specific aims: i) demonstrate the morphology of the ehrlichia-host cell membrane interaction in HL60 cells in various differentiated states; ii) characterize the role of E. phagocytophila group MSPs as adhesins and the role of CD15-associated structures as the major surface receptors by which ehrlichiae attach and enter cells; iii) characterize the effects of ehrlichial binding and internalization on host granulocyte cell surface adhesion molecule expression, granulocyte-endothelial cell adherence, phagocytic activity, activation/deactivation, microbial killing, and cytokine/chemokine expression; and iv) identify the chromosomal ligand for the E. phagocytophila group ankyrin protein, EPANK1.

Grant: 1R01AI044103-01A1
Program Director: KLEIN, DAVID L
Principal Investigator: JARVIS, GARY A
Title: IMMUNOLOGY OF BACTERIAL PNEUMONIA IN HTLV-II INFECTION
Institution: NORTHERN CALIFORNIA INSTITUTE RES & SAN FRANCISCO, CA
EDUC
Project Period: 2000/03/01-2003/02/28

DESCRIPTION (Adapted from the Applicant's Abstract): This projects seeks to understand the immunologic basis for the epidemiological outcome observation of an increased incidence of bacterial pneumonia in HTLV-II-infected blood donors. In a study of persons with both HTLV-II seropositivity and intravenous drug use, an increased risk for bacterial pneumonia compared to patients with neither risk was found. A similar finding of increased risk for bacterial pneumonia in HTLV-II-infected individuals was observed in a clinical outcome analysis of blood donors who participated in a Retroviral Epidemiological Donors Study. Since the cell tropism of HTLV-II infection includes CD4+ and CD8+ T cells, B cells and monocytes, the investigators will determine whether HTLV-II infection predisposes to bacterial pneumonia by creating immune dysfunction in these cells which are known to be critical for natural protection against pneumococcal pneumonia, the most common cause of bacterial pneumonia in individuals with immune dysfunction. Studies planned for the next five years address the immune status of HTLV-II-infected persons. HTLV-II-infected and -uninfected individuals will be vaccinated with the pneumococcal 23-valent polysaccharide vaccine, and their qualitative and quantitative antibody responses will be investigated by determining the isotype and IgG subclass distribution, concentration, avidity and opsonic function of specific pneumococcal capsular antibodies. The same individuals will also be vaccinated with tetanus toxoid protein antigen as a control for antibody response to a separate class of antigen, and their antibody responses measured. The effect of HTLV-II infection of T cells on the proliferative and antibody-secreting functions of B lymphocytes will be tested. CD4+ and CD8+ T cells will be isolated from HTLV-II-infected persons using immunobeads, co-cultivated with normal B lymphocytes, and the functional activity of the B cells assessed using mitogen stimulation and ELISA antibody quantitation assays. Levels of B cell regulatory cytokines produced by infected and uninfected T cells will be measured by ELISA. Levels of immune response markers IL2 receptor on CD8+ T cells, and CD21 and CD35 on B cells will be determined by flow cytometry. The phagocytic and bactericidal function of PMN and of human macrophages derived from the monocytes from HTLV-II-infected individuals will be tested and compared to the function of those cells from uninfected persons. Human CD4+ and CD8+ T cells will be isolated using immunobeads and quantities of macrophage and PMN regulatory cytokines produced by HTLV-II-infected and -uninfected T Cells will be measured by ELISA. The effect of HTLV-II infection of human T cells on cytokine-dependent activation of macrophages and PMN will be studied using functional assays of pneumococcal phagocytic killing.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01AI044148-01A1
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: ENGEL, JOANNE N MD MEDICINE
Title: BIOGENESIS OF THE CHLAMYDIA TRACHOMATIS VACUOLE
Institution: UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA
Project Period: 2000/02/01-2005/01/31

DESCRIPTION (Adapted from the Applicant's Abstract): Chlamydia trachomatis is the leading cause of sexually transmitted diseases in this country and a major cause of blindness in third world countries. The ability of this obligate intracellular parasite to enter a non-phagocytic epithelial cell and survive within the hostile intracellular environment of the eukaryotic cytoplasm is key to its pathogenesis. The intimate interactions between chlamydia and its eukaryotic host is likely to involve natural biological pathways of the eukaryotic cell that the parasite usurps for its own survival. Study of these processes will yield insights into eukaryotic cell biology as well as insights into chlamydial disease pathogenesis. From these studies may emerge new therapeutic approaches to treating or preventing chlamydial infections. Specific Aim 1: The investigators hypothesize that successful *C. trachomatis* biovar LGV entry and intracellular development in epithelial cells involves at least two separate pathways, one of which is clathrin-independent, and have preliminary evidence that entry and/or development is dependent upon the host actin cytoskeleton and is modulated by c-src. (A) They will test the role of clathrin mediated endocytosis by assessing the effect in epithelial cells of expression of dominant negative (DN) alleles of dynamin, ARF-6, or clathrin on *C. trachomatis* binding, entry, and replication. (B) They will further investigate the role of the actin cytoskeleton in the *C. trachomatis* life cycle by determining whether the actin-regulating GTPases rac, rho, and CDC42 affect LGV and serovar E binding, entry, and replication in polarized and non-polarized epithelial cells. (C) They will determine the mechanism of c-src-mediated stimulation of *C. trachomatis* infectivity. Specific aim 2: An unusual aspect of the *C. trachomatis* life cycle is the receipt of sphingomyelin from the trans Golgi Network (TGN) by the bacteria-containing vacuole. They will test the hypothesis that the *C. trachomatis* vacuole interacts with one or more apical exocytic pathways including the newly proposed exocytic pathway in which lipid rafts transport sphingolipids, glycosylphosphatidylinositol (GPI)-anchored proteins, and other designated proteins to the apical surface of polarized epithelial cells. Using several approaches, they will identify specific host cell factors required for the delivery of sphingomyelin from the TGN to the *C. trachomatis* vacuole. This will help to further define the pathway involved. These studies may lead to the development of new anti-chlamydial drug therapies and further our understanding of lipid trafficking in eukaryotic cells.

Grant: 1R01AI044235-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: DAVIDSON, WILLIAM R
Title: HUMAN EHRLICHIOSIS SURVEILLANCE AND EPIDEMIOLOGY
Institution: UNIVERSITY OF GEORGIA ATHENS, GA
Project Period: 2000/08/01-2004/07/31

DESCRIPTION (Adapted from the Applicant's Abstract): Two tick-borne ehrlichioses, human monocytic ehrlichiosis (HME) caused by *Ehrlichia chaffeensis* and human granulocytic ehrlichiosis (HGE) caused by *Ehrlichia equi* or a closely related organism, have emerged in the United States since 1986. The long-range goal of this research is to enhance the diagnosis, prevention, and control of human ehrlichioses by determining their geographic distributions, epidemiologic risk factors, and genetic relationships. Deer are intimately linked to HME and HGE because they serve as reservoirs, key hosts for tick vectors, or both. Deer harbor a third potentially zoonotic but un-described *Ehrlichia*. The rationale for the proposed research is to exploit the unique role that deer play in the natural history of these ehrlichiae to accomplish a major objective of elucidating the landscape and molecular epidemiology of these pathogens. To accomplish this objective, we will pursue five specific aims: (1) develop and implement a surveillance system utilizing deer as natural sentinels to delineate the geographic distributions of *E. chaffeensis*, the HGE agent, and the deer *Ehrlichia* organism;(2) experimentally evaluate the suitability of deer as a reservoir for the HGE agent;(3)isolate and characterize the deer *Ehrlichia* organism in order to facilitate development of diagnostic assays required to test humans for infection by this agent;(4)determine the molecular variation of strains of *E. chaffeensis* and the HGE agent from deer populations and compare this genetic variability with isolates from humans; and,(5)utilize geographic information system(GIS)technology and spatial analyses to relate the distributions of *E. chaffeensis*, the HGE agent, and the deer *Ehrlichia* to ecological variables and compare these landscape epidemiologic patterns with the reported distributions of HME and HGE cases. When completed this research should:(1)provide a better estimate of the relative risk of infection in different areas;(2)identify areas where increased diagnostic or preventive efforts are needed;(3) provide data useful for designing more powerful epidemiologic or risk assessments; and, (4) provide biologic material for improved diagnostic assays.

Grant: 1R01AI044240-01A2
Program Director: BAKER, PHILLIP J.
Principal Investigator: HU, LINDEN T MD
Title: PEPTIDE TRANSPORT AND SIGNALING IN BORRELIA BURGENDORFERI
Institution: NEW ENGLAND MEDICAL CENTER BOSTON, MA
HOSPITALS
Project Period: 2000/03/01-2005/02/28

DESCRIPTION (Adapted from the Applicant's Abstract): One of the striking observations that has been made about *Borrelia burgdorferi* in the post-genomic era is its apparent lack of synthetic machinery. *B. burgdorferi* does not appear to have any genes for the synthesis of amino acids, fatty acids, enzymes cofactors or nucleotides. As a result, the organism is dependent upon its environment to supply these essential nutrients. Many bacteria possess multiple peptide transport systems with different specificities to facilitate the utilization of peptides as a source for amino acids. The *B. burgdorferi* genome appears to encode for only a single putative peptide transport system. However, unlike many other bacteria which have only a single peptide binding protein for each transport system, it appears that *B. burgdorferi* encodes for up to 5 peptide binding proteins (OppA-1 to 5) which may share the same integral membrane transport (OppBC) and ATP-binding machinery (OppDF). The *B. burgdorferi* putative transport system has a high degree of identity to the Opp and Dpp transport systems of *E. coli*. Using toxic peptide substrates which are known to be taken up by these systems in *E. coli*, Dr. Hu and coworkers have shown that *B. burgdorferi* is able to transport peptides from its environment. They have also performed studies in opp of *E. coli* where the deleted operon is replaced by plasmids expressing *B. burgdorferi* OppA-1 and *E. coli* OppBCDF or *E. coli* OppA and *B. burgdorferi* OppBCDF. Using toxic peptides to inhibit the growth of the bacteria, they have shown that *B. burgdorferi* Opp proteins are capable of complementing their *E. coli* counterparts to transport peptides. The central hypothesis of this project is that *B. burgdorferi* uses its 5 substrate binding proteins to broaden substrate specificity and compensate for its paucity of peptide transporters. The first goal of the project will be to test this hypothesis by defining the substrate specificities of each *B. burgdorferi* OppA and subtype. In addition to their nutritional role, the peptide transport systems of other bacteria play important roles in environmental signaling with diverse interactions ranging from quorum sensing to peptide chemotaxis. Very little is currently known about what stimulates *B. burgdorferi* to multiply and move from the tick gut to the salivary glands and into the mammalian host as the tick takes its blood meal. Dr. Hu's group believes that the Opp system of *B. burgdorferi* may be part of a coordinated response of the organism to environmental shifts as it moves from its tick to mammalian hosts and back again. They will test this hypothesis by examining changes in expression of the various Opp proteins as the organism moves from host to host. They will also test the hypothesis that one or more of the OppA proteins of *B. burgdorferi* may be involved in chemotaxis of the organism. This group believes that a better understanding of the peptide transport system of *B. burgdorferi* will broaden existing knowledge about how the organism adapts to its multiple hosts as well as the role that these adaptations play in the pathogenesis of Lyme disease, and may eventually lead to the development of novel therapeutics in the treatment of Lyme disease.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01AI044254-01A2
Program Director: BAKER, PHILLIP J.
Principal Investigator: STEVENSON, BRIAN PHD
Title: ANALYSIS OF BORRELIA BURGENDORFERI ERP PROTEINS
Institution: UNIVERSITY OF KENTUCKY LEXINGTON, KY
Project Period: 2000/03/01-2005/02/28

The spirochete *Borrelia burgdorferi* is the causative agent of Lyme disease. Infected mammals produce bacteriocidal antibodies against *B. burgdorferi* surface proteins, and passive transfer of sera from infected humans and laboratory animals can protect naive animals against *B. burgdorferi* challenge. Yet antibodies directed against *B. burgdorferi* often cannot effectively clear infection, and bacteria can persistently infect humans and other mammals with periodic recurrence of symptoms. These observations suggest that *B. burgdorferi* alters its surface properties during mammalian infection to evade clearance by the host immune system. Consistent with this prediction, it is well known that *B. burgdorferi* can regulate the synthesis of antigenic surface proteins, both in vivo and in vitro. Within the first four weeks of infection, mammals produce antibodies against the *B. burgdorferi* Erp lipoproteins, indicating that the bacteria synthesize Erp proteins during this time period. It is proposed that Erp proteins are involved in the initial infection process by enabling *B. burgdorferi* to be transmitted from the tick vector and/or interact with mammalian tissues. Infected mammals consistently produce antibodies directed against Erp proteins, but can still be chronically infected by *B. burgdorferi*. As a corollary to the central hypothesis, it is predicted that Erp proteins are not produced during the later stages of mammalian infection. *B. burgdorferi* can regulate the synthesis of Erp proteins in culture, and it is hypothesized that Erp protein production is also regulated by similar mechanisms in vivo. The specific aims of the proposed studies are: (1) Characterize erp genes and their proteins in cultured bacteria by investigating the in vitro differential expression of erp mRNAs and proteins, the ability of individual bacteria to simultaneously express their entire repertoire of Erp proteins, the surface accessibility of each Erp protein, and the ability of antibodies directed against Erp proteins to inhibit *B. burgdorferi* growth. (2) Analyze erp genes and their proteins during mammal and tick infections by examining levels of erp transcripts and Erp proteins throughout the mammal-tick infectious cycle. (3) At this time, essentially nothing is known about the regulatory mechanisms that govern expression of the erp genes or any other *B. burgdorferi* gene. Cultured bacteria will be used to further characterize mechanisms of erp gene regulation, including the continued purification of a *B. burgdorferi* protein that specifically binds to erp promoter DNA.

Grant: 1R01AI044918-01A1
Program Director: LANG, DENNIS R
Principal Investigator: LYTE, MARK
Title: NEUROENDOCRINE MEDIATION OF E. COLI O157:H7 INFECTION
Institution: MINNEAPOLIS MEDICAL RESEARCH FDN, MINNEAPOLIS, MN
INC.
Project Period: 2000/04/01-2004/03/31

This application proposes the new theory of directed neuroendocrine- bacterial interactions as a mechanism governing the ability of an enteric pathogen to infect a host. This hypothesis is based on the in vitro and in vivo ability of the neuro-endocrine hormone norepinephrine (NE) to increase growth and production of virulence-associated factors of the enteric pathogen Escherichia coli O157:H7. High concentration of NE occur in foods such as ground beef which are contaminated by E. coli O157:H7. Equally high concentrations of NE also occur within the gastrointestinal tract due to enteric nervous system activity. The proposed research will therefore examine whether the presence of NE from the time of E. coli O157:H7 contamination of NE-rich foods to infection within the gut may be a factor mediating the development of hemorrhagic colitis. Results from this laboratory have shown that the effect of NE on E. coli O157:H7 contamination of NE-rich foods to infection within the gut may be a factor mediating the development of hemorrhagic colitis. Results from this laboratory have shown that the effect of NE on E. coli O157: H7 is due to the production of an autoinducer of growth. Thus, our Specific Aims are: 1) To determine the ability of a purified diet supplemented with levels of NE found in commonly contaminated foods to "prime" E. coli O157:H7 for the NE-rich environment within the gastrointestinal system; 2) To examine the ability of E. coli O157:H7 isolated from gastrointestinal tract of stressed and non-stressed mice since differences in luminal levels of NE between stressed and control animals would provide greater understanding of the recognized ability of stress to alter susceptibility to colitis; 4) To determine the ability of stress of alter the susceptibility of mice to oral challenge with E. coli O157:H7 exposed in vitro to control of NE supplemented diets; 5) To examine whether blockage of NE release within the gastrointestinal tract can alter susceptibility to challenge with E. coli P157:H7; and 6) To purify the serum-bound form of the NE-induced E. coli O157:H7 autoinducer of growth and determine its structure which may provide the basis for the development of agents to specifically interrupt bacterial division as well as identify the gene(s) involved in its production. Collectively, the above aims will seek to establish a direct cause and effect relationship between the NE content within food and the gastrointestinal tract to influence the ability of E. coli O157:H7 to cause infection. The demonstration of direct neuroendocrine-bacterial interaction as a mechanism in the pathogenesis of E. coli O157:H7 infection may yield new treatments for both the prevention and treatment of hemorrhagic colitis.

Grant: 1R01AI044935-01A2
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: BARNES, PETER F
Title: TRANSMISSION DYNAMICS OF TB ALONG THE U.S.-MEXICO BORDER
Institution: UNIVERSITY OF TEXAS HLTH CTR AT TYLER, TX
TYLER
Project Period: 2000/09/15-2005/08/31

DESCRIPTION (Adapted from the Applicant's Abstract): To devise more effective means to reduce spread of tuberculosis (TB) in the U.S., it is critical to understand the transmission dynamics of drug-resistant and drug-susceptible TB in different socioeconomic settings, including small cities, rural areas and locations where foreign-born persons enter the U.S. To address these issues, the investigators propose a population-based molecular epidemiologic study of TB along the U.S.-Mexico border. They hypothesize that: (1) the transmission dynamics of TB in small cities and rural areas differ from those in large cities; (2) Prospective use of molecular epidemiology will delineate the transmission dynamics of drug-susceptible and drug-resistant TB; (3) A rapid method of RFLP analysis of M. tb isolates will enhance timely detection of TB outbreaks. Our specific aims are-1. To determine the nature of epidemiologic links between patients who are infected with the same M. tb strain, and to identify locations and settings where TB is transmitted, including an analysis of transmission across the U.S.-Mexico border. This will be achieved by correlating detailed clinical and epidemiologic data on TB patients with results of RFLP analysis of M. tb isolates. 2. To characterize the development and transmission dynamics of drug-resistant TB, including comparison of the transmission potential of M. tb isolates with or without katG mutations. This will be achieved by evaluation of drug susceptibility patterns, clinical and epidemiologic data, RFLP results and sequencing of the katG gene. 3. To evaluate the utility of spoligotyping, a rapid method of RFLP analysis, to identify unsuspected TB outbreaks and guide public health interventions to reduce TB transmission. Spoligotyping will be adapted for use in clinical samples and early mycobacterial cultures, then prospectively applied to all M. tb isolates. This study will provide insight into the transmission dynamics of TB in mixed urban/rural areas. It will evaluate the relationship between katG mutations and transmission potential, providing clues to the mechanisms underlying mycobacterial virulence. Finally, it will determine if rapid RFLP methods can contribute to TB control efforts. The knowledge gained will permit refinement of TB control strategies along the U.S.-Mexico border and in Latin America.

Grant: 1R01AI044954-01A1
Program Director: LANG, DENNIS R
Principal Investigator: FAN, ERKANG PHD
Title: MULTIDENTATE HIGH AFFINITY LIGANDS FOR AB5 TOXINS
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2000/02/01-2005/01/31

The goal of this proposal is to take advantage of the structural symmetry of multimeric proteins, a rarely explored property, to arrive eventually at structurally complementary multidentate protein ligands with ultra-high affinity and specificity. The long term objective is to illuminate an area of fundamental biological interest: the molecular recognition properties of multidentate protein ligands and the use of such ligands to control protein functions. Specifically, this proposal encompasses the design, synthesis and evaluation of multidentate ligands targeting a pair of ideal model systems: the heat-labile enterotoxin from *E. coli* (LT) and the closely related cholera toxin secreted by *V. cholerae* (CT), which are both AB₅ heterohexamers. The biological mechanism of the actions of LT and CT includes a critical step of receptor recognition on the target cell by the B pentamer. The five-fold symmetry of the B subunits of LT and CT offers good opportunities to develop pentadentate ligands with overall structures complementary to the arrangement of toxin receptor binding sites. Such ligands will be created stepwise using a modular approach with each module providing opportunities for further optimization. Building on the principles of molecular recognition, our proposed work will combine the powers of combinatorial chemistry and structure-based design to arrive at ultrahigh affinity pentadentate ligands. The affinity of the ligands obtained will be investigated with a variety of analytical tools. Detailed thermodynamics of ligand-protein interaction will be studied using a series of mono- to penta-dentate ligands. The proposed research has broad implications for the field of molecular recognition in general since it is at the frontiers of investigations focusing on multidentate ligands interacting with multimeric proteins. In addition, high affinity ligands derived from our work have potential health benefits, as they may lead to the development of agents useful for the detection, treatment, and prevention of AB₅ toxin-related enterotoxigenic diseases.

Grant: 1R01AI045041-01A2
Program Director: MILLER, MARISSA A.
Principal Investigator: HURLBURT, BARRY K
Title: MECHANISMS OF VIRULENCE GENE REGULATION IN *S. AUREUS*
Institution: UNIVERSITY OF ARKANSAS MED SCIS LTL LITTLE ROCK, AR
ROCK
Project Period: 2000/09/27-2001/05/31

DESCRIPTION (Adapted from the Applicant's Abstract): Despite intensive research efforts over the past 50 years, *Staphylococcus aureus* remains a serious threat to human health. In fact, recent reports of isolates with reduced susceptibility to vancomycin are indicative of the growing likelihood that *S. aureus* may become resistant to every currently available antimicrobial agent. Therefore, *S. aureus* represents a bigger threat to human health now than at any time since the pre-antibiotic era. The long-term goal of the investigators' research is to develop novel anti-staphylococcal therapeutic agents to stem the advance of drug-resistant strains. The current proposal is focused on the interaction of two globally-acting regulators of virulence gene expression: *sar* and *agr*. Previous work by the investigators and others indicate that the product of the *sar* gene, SarA, is a transcription factor that activates *agr* expression and leads to enhanced virulence of the organism. Because *agr*, *sar* and *agr/sar* mutants are less virulent in animal models of staphylococcal disease, they believe that the SarA/*agr* interaction is a very promising target for novel inhibitors of staphylococcal virulence. Importantly, since *sar* and *agr* mutants grow normally, but are less virulent, there should be reduced evolutionary pressure to become resistant to this type of inhibitor in comparison with classical antibiotics that inhibit growth. In addition, there are reports of increased sensitivity of *sar* and *agr* mutants to existing antimicrobial agents including methicillin. Inhibitors of the SarA/*agr* interaction may therefore be useful with respect to increasing the utility of existing drugs. To provide the foundation for inhibitor development, they will first reveal the mechanism by which SarA activates *agr* gene expression. The first three Specific Aims of the proposed research are: 1) to define the cis regulatory elements responsible for activation of the *agr* genes by SarA, 2) to define the functional domains of SarA required for activation of *agr* transcription, 3) to define the interaction of SarA with the *agr* regulatory elements at the atomic level. These aims will be accomplished by a combination of mutagenesis, in vitro and in vivo activity assays, x-ray crystallography, and atomic force microscopy. The fourth Specific Aim, to begin to develop anti-staphylococcal inhibitors that target these global regulatory factors, is intended to test the hypothesis that SarA-*agr* is a suitable target for novel anti-staphylococcal drugs. This broad-based approach takes advantage of a coordinated team, each member of which is an expert in his/her field.

Grant: 1R01AI045407-01A2
Program Director: LAUGHON, BARBARA E.
Principal Investigator: VON REYN, C FORDHAM
Title: DISSEMINATED TUBERCULOSIS IN HIV INFECTION
Institution: DARTMOUTH COLLEGE HANOVER, NH
Project Period: 2000/08/01-2005/07/31

Disseminated infection (mycobacteremia) with *Mycobacterium tuberculosis* (dMTB) has been documented by our group in 10-25 percent of patients with HIV infection in Africa using lysis-centrifugation blood cultures. Unlike pulmonary tuberculosis (pMTB), most cases of dMTB are not recognized and death ensues rapidly. Thus, in developing countries dMTB may be a more important cause of HIV-associated mortality than pMTB. Risk factors for dMTB have not been identified and it is not known if most cases are due to primary infection, reactivation or re-infection. We hypothesize that most cases of dMTB are due to primary MTB infection in patients without prior infection with MTB or non-tuberculous mycobacteria (NTM). Mycobacterial immunization in early HIV infection is a potential strategy to prevent dMTB. *Mycobacterium vaccae* (MV) is an investigational vaccine prepared by heat inactivation of an NTM, and has been shown to be protective against MTB in several animal models. Studies conducted by our group indicate that a 5-dose series of MV is safe in patients with HIV infection and induces a durable cellular immune response to MTB antigens in persons with prior BCG immunization. Our hypothesis is that MV immunization will reduce the risk of HIV-associated dMTB by 50 percent. Our specific aims are: (1) to define risk factors for HIV-associated disseminated tuberculosis and to assess the relative contributions of primary infection, reactivation and re-infection in the pathogenesis of disseminated tuberculosis, and (2) to assess the safety and efficacy of a 5-dose schedule of inactivated MV vaccine for the prevention of HIV-associated pulmonary and disseminated tuberculosis in persons with prior BCG immunization. 2274 HIV-positive patients with prior BCG immunization and 100 HIV-negative controls will be entered in a 5-year study in Zambia. Baseline evaluation will include history, chest x-ray, dual skin tests with purified protein derivative (PPD) and *Mycobacterium avium* sensitin (MAS), and whole blood assay for interferon-gamma production in response to MV sonicate, PPD, ESAT-6 (a protein antigen unique to MTB) and MTB antigen 85. Subjects with PPD reactions greater than or equal to 5 mm will receive 6 months of prophylaxis with isoniazid. All subjects will be randomized 1:1 to receive a 5-dose series of MV or placebo over 12 months with repeat skin test and in vitro studies at 14 months. Subjects will be followed every 3 months for 3-5 years to assess new pMTB (microbiologic or clinical diagnosis) or dMTB (microbiologic diagnosis). All isolates will have susceptibility tests and IS6110 DNA fingerprinting performed. Potential risk factors for dMTB, including baseline PPD test results, will be assessed in placebo and vaccine groups. Vaccine efficacy against dMTB and pMTB in HIV-positive subjects will be determined, and post immunization interferon gamma responses used to identify a surrogate marker of efficacy. The proposed study has important implications for the reduction in mortality from HIV-associated tuberculosis and for design of future trials of new vaccines against tuberculosis.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01AI045459-01A2
Program Director: LAUGHON, BARBARA E.
Principal Investigator: PIROFSKI, LIISE-ANNE MD
Title: VARIABLE GENE USE AND PNEUMOCOCCAL IMMUNITY IN AIDS
Institution: YESHIVA UNIVERSITY NEW YORK, NY
Project Period: 2000/07/01-2005/06/30

DESCRIPTION (adapted from abstract of applicant): Human antibodies to capsular polysaccharide of *Streptococcus pneumoniae* (PPS) express genes from the VH3 gene subgroup. Since VH3 gene expression is often reduced in the setting of HIV infection and PPS-derived vaccines are poorly immunogenic in HIV-infected individuals, we have hypothesized that decreased expression of the immunoglobulin genes used in antibodies to PPS contributes to PPS vaccine failure in the setting of HIV infection. The structure-function relationship of human antibodies to most pneumococcal serotypes has not been fully investigated. The studies that are proposed in this application are designed to characterize the variable gene use and epitope specificity of human antibodies to two common serotypes of *S. pneumoniae*, serotypes 8 and 23F, and to use this information to develop probes to determine the specificity of vaccine elicited antibodies to PPS 8 and 23F from individuals with and without HIV infection. The specific aims are: 1) To determine the molecular structure of human Mab to PPS 8 and 23F generated in transgenic mice reconstituted with human immunoglobulin loci; 2) To characterize the Mabs to PPS 8 and 23F produced in aim one as protective, non-protective or disease enhancing against pneumococcal infection in mice and as opsonic in vitro; 3) To use Mabs with functional efficacy as defined in aim two to isolate peptide mimetics of PPS 8 and 23F epitopes, and to use the peptides to determine if PPS-elicited antibodies in patients sera recognize protective, non-protective or disease enhancing epitopes. Studies are proposed to examine the hypothesis that protective antibodies to PPS can be distinguished by their variable gene use and specificity and that the production of antibodies to certain epitopes, which are derived from VH3 genes, is impaired in the setting of HIV infection. Knowledge gained from these studies will help us to understand the structure-function relationship for antibodies to *S. pneumoniae* in patients with HIV infection, and to identify possible correlates of PPS vaccine failure and the requirements for more effective anti-pneumococcal vaccines. Significance: Infection with pneumococcus has become a major cause of morbidity and mortality in AIDS. In the past, AIDS-associated infections were easily treated with antibiotics however, penicillin resistant isolates have emerged in the 1990's. This emergence of antibiotic resistant pneumococcus has increased the need for vaccination of high-risk groups such as HIV+ persons. However, available pneumococcal vaccines are poorly immunogenic in HIV+ persons. Thus, the goal of this proposal, which is to identify the molecular and biological basis of human antibody responses to pneumococcal vaccination in normal volunteers and HIV+ persons, is very significant. The information gained from the proposed studies could lead to better vaccines for pneumococcus in immunocompromised hosts and importantly, will contribute to the understanding of how antibodies function in resistance to *S. pneumoniae*. Approach: This is the second revision of this application. Since the last review of this application the author has published 1 manuscript and has two others in press having to do with this application. The first publication describes the generation of a protective human Mab to PPS 8. The second, describes the generation of human

Includes Research Project Grants (RPGs)
Excludes clinical trials

antibodies to PPS 3 in XenoMouse mice. The third describes the molecular response of HIV-infected and HIV-uninfected subjects to Pneumovax. Thus, the author has made excellent progress even though she is not funded to do this work. This indicates commitment, enthusiasm and capability of the author to carry out this work. The P.I. has responded to the previous criticisms and has written a strong and significant application. One previous concern was that if HIV-infected individuals can not produce VH3 and only VH3 ab are protective that little of this information will be useful to make patients more resistant to pneumococcus. The PI points out that the goal of this application is not to characterize VH3 expression in HIV-infected individuals, but to determine whether they do or do not produce antibodies to pneumococcal capsular polysaccharides with defined epitope specificity. The PI's response and revisions to other reviewer's comments also strengthen the 1 ZRG-1 AARR- 4 (01) 3 1 R01 AI45459-01A2 March 2000 Pirofski, Liise-Anne proposal. In addition, because of the subject of this application, the initial applications were difficult to follow. This revised version is clearer and the additions the PI has made are helpful. It is much more apparent what the PI really wants to accomplish. This is important since the reviewers agreed that this was a difficult application to read, mainly because of the subject matter and the specialized nature of the work. Innovation: The overall approach utilized in solving this problem takes advantage of established methods. The author has used these methods in the study of Cryptococcus so that the overall strategy is somewhat proven. Use of the XenoMouse is considered innovative. There are relatively few laboratories interested in antibody-mediated resistance. This makes this work somewhat unique. Investigator: Dr. Pirofski is well qualified to carry out the proposed research. She has published studies utilizing most of the methods in the proposal. Dr. John McKittrick is a consultant and brings expertise in the microbiology of *S. pneumoniae*. Environment: The P.I. is at Albert Einstein and has several other researchers nearby that have expertise in many of the methods she will utilize. She is in an excellent environment to accomplish this proposal. In summary, the subject of this proposal is timely and significant. The emergence of antibiotic resistant *S. pneumoniae* infections in AIDS patients requires the development of more efficient means of immunizing these individuals. The PI has made excellent progress on this project despite not being funded in this area. Studies proposed in this application could provide valuable information for developing more immunogenic vaccines for HIV+ persons. Simply put, this work needs to be done.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01AI045569-01A1
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: OLSON, JOAN C PHD
Title: MODULATION OF CELL SIGNALING BY PSEUDOMONAS EXOENZYME S
Institution: MEDICAL UNIVERSITY OF SOUTH CHARLESTON, SC
CAROLINA
Project Period: 2000/05/01-2004/04/30

DESCRIPTION (Adapted from the Applicant's Abstract): Exoenzyme S (ExoS) is an ADP-ribosylating toxin produced by the opportunistic pathogen *Pseudomonas aeruginosa* which requires direct contact between the bacterium for translocation into the host cell. Once within the target cell, ExoS causes complex effects on cellular processes resulting in inhibition of DNA synthesis and alterations in the cytoskeleton affecting cell structure, movement, microvilli and focal adhesion turnover. Studies from the PI's laboratory have led to the development of a bacterial-eukaryotic cell co-culture system which allowed the toxic effects of ExoS to be identified. This system was also used to provide insight into the cellular mechanism of action of ExoS by identifying Ras as an in vivo target of ExoS ADP-ribosyltransferase (ADPRT) activity. The complex effects of ExoS on cellular function can to some extent be explained by its multi-domain structure. Current data support the possibility that ExoS can cause transient alterations in cytoskeletal structure via non-ADPRT mechanism which is then coordinated with the ADP-ribosylation of specific cellular proteins leading to long-term alterations in cytoskeletal structure and inhibition of DNA synthesis. The cellular mechanism for the diverse affects of ExoS on cell function, however, remains unknown, and may relate to the ADP-ribosylation of cellular Ras, which plays an integral role in multiple signal transduction pathways, the ADP-ribosylation of other cellular proteins, or to indirect effects of ExoS on proteins linked to the cytoskeleton. The goal of this proposal is to identify cellular processes and signaling pathways affected by ExoS following bacterial translocation. The purpose of the first specific aim is to gain further understanding of cellular proteins directly affected by ExoS by examining the in vivo substrate specificity of ExoS ADPRT activity. The second aim focuses on Ras, examining how ADP-ribosylation of Ras by ExoS affects Ras mediated cell signaling events. The third aim examines the cellular mechanism of ExoS associated alteration in cytoskeletal structure, focusing on both enzymatic and non-enzymatic effects of ExoS on Rho, Rac, and Cdc42, which function in the regulation of cytoskeletal structure, the combined picture will provide insight into how ExoS influences cellular signaling, and in turn, the role of ExoS in *Pseudomonas* pathogenesis.

Grant: 1R01AI045602-01A1
Program Director: MILLER, MARISSA A.
Principal Investigator: HEINZEL, FREDERICK P MD
Title: ENDOTOXIN TOLERANCE AS A MODEL FOR IMMUNE PARALYSIS
Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH
Project Period: 2000/03/15-2004/02/28

DESCRIPTION (adapted from applicant's abstract) The applicants propose to study the pathogenesis and biologic significance of injury-induced defects in the innate cellular immune response. These include studies of both experimental endotoxin tolerance in mice and clinical immune paralysis in cardiac surgery patients. Endotoxin tolerance is induced by repeated exposures to lipopolysaccharide (endotoxin) and results in reduced synthesis of pro-inflammatory cytokines to microbial stimuli. Immune paralysis is phenotypically similar, but is a clinical phenomenon induced by sepsis or trauma. They hypothesize that endotoxin tolerance and immune paralysis disrupt the interdependent production and synergistic anti-microbial activities of TNF-alpha, IL-12 and IFN-gamma that mediate the innate cellular immune response. Their first goal is to use the well-characterized mouse model of endotoxin tolerance to identify mechanisms that mediate the immune defects of endotoxin tolerance and enhance susceptibility to infection. They will then identify interventions that prevent or reverse these immune deficiencies. Preliminary data already indicate immune cell depletion is a mechanism for injury-induced cytokine deficiency and identify a 10- to 10,000-fold enhanced susceptibility to candidiasis during endotoxin tolerance. The applicants' second goal is to study the biologic basis and epidemiologic consequence of immune paralysis in cardiac surgery patients, with a long term goal of developing clinical interventions to prevent immune paralysis. The specific aims of this proposal are: 1) Identify molecular and/or cellular defects in the innate immune response of endotoxin tolerant mice. 2) Determine if the defective innate immunity of endotoxin tolerance enhances susceptibility to common nosocomial pathogens. 3) Use these findings to rationally design drug or cytokine therapies that prevent the immune defects of endotoxin tolerance and thereby reduce susceptibility to nosocomial superinfection. 4) Characterize comparable cytokine and cellular defects in hospitalized patients following cardiac surgery and identify immune phenotypic markers predictive of post-operative infections. Studies of the immune pathogenesis of infectious susceptibility in endotoxin tolerant mice are likely to suggest cytokine- or anti-apoptosis-based therapies for clinical immune paralysis. This is a clinically desirable goal, as the preservation or enhancement of innate immunity against nosocomial infections in injured patients may significantly reduce hospital morbidity, costs, antibiotic use and the selection of antibiotic-resistant pathogens.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01AI045642-01A1
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: GRANOFF, DAN M MD
Title: MIMETICS OF CAPSULAR EPITOPES OF NEISSERIA MENINGITIDIS
Institution: CHILDREN'S HOSPITAL & RES CTR AT OAKLAND, CA
OAKLAND
Project Period: 2000/04/01-2005/03/31

Description (Adapted from the applicant's abstract) The long-term objective of this study is to increase our understanding of the immunologic properties of molecular mimetics of a bacterial polysaccharide that also is an autoantigen. Mimetics can act as antigens, bind to antibody receptors on B cells, and elicit serum antibody responses to the nominal antigen. Yet, little is understood about the properties of antigenic mimetics that contribute to their immunogenicity. Nor is much known about the quality of the antibody response with respect to conferring protection against a pathogen. *Neisseria meningitidis* group B (NmB) polysaccharide is an excellent model to investigate these questions. NmB is a major cause of meningitis and sepsis. The mechanism of protection is well defined (serum antibody), and reliable assays exist for measurement of antibody binding to the polysaccharide antigen, functional antibody against the bacteria, and passive protection in an infant rat model of bacteremia. Efforts to develop a NmB vaccine have been hampered by poor immunogenicity of the polysaccharide capsule, which cross-reacts with host polysialic acid. The PI's laboratory has prepared a panel of murine monoclonal antibodies (Mabs) that react with capsular polysaccharide epitopes on NmB that are distinct from host polysialic acid. These Mabs are bactericidal and confer passive protection in animal models. The PI's hypothesis is that mimetic antigens identified by these Mabs will be able to elicit protective anti-capsular antibody responses that are specific for the pathogen, avoiding the risk of autoantibody. Further, that the best mimetics will be those that adopt relatively stable conformations. To search for such mimetics, the PI proposes to screen phage libraries displaying independently folding peptides, or search for constrained protein mimetics within immunoglobulin scaffolds (single chain variable (ScFv) anti-idiotypes). The mimetics will be investigated for their ability to elicit anticapsular antibody responses. The Ig isotype, antibody functional activity to the bacteria, and immunoglobulin variable region gene usage in response to the mimetics, will be compared to that elicited by the nominal antigen. A central question will be whether a mimetic identified with a Mab that reacts specifically with encapsulated NmB strains, but not with host polysialic acid, will elicit protective antibody responses without autoantibody activity. Additional questions will be whether the repertoire of the antibody response to the mimetic is similar or different than that elicited by the nominal polysaccharide, and what effect, if any, the antibody repertoire may have on protection. Taken together, the data will provide important information on the properties of mimetic antigens with chemical structures that are distinct from that of the nominal polysaccharide antigen. The results also may identify new candidate molecules for inclusion in a future vaccine for prevention of NmB disease.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01AI045658-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: MCDONOUGH, KATHLEEN A PHD
Title: GENE EXPRESSION OF M.TUBERCULOSIS WITHIN MACROPHAGES
Institution: WADSWORTH CENTER ALBANY, NY
Project Period: 2000/07/01-2005/06/30

DESCRIPTION (Adapted from the Applicant's Abstract): The long term objective of this proposal is to gain a better understanding of how Mycobacterium tuberculosis establishes infection so that effective strategies can be developed to prevent it. A multidisciplinary approach will focus on the interaction of tubercle bacilli with macrophages at the molecular, genetic and cellular levels, with an emphasis on M. tuberculosis gene expression within macrophages. The specific aims are: 1) Identifying M. tuberculosis genes that are induced when bacteria are within macrophages by 2D gel electrophoresis coupled with mass spectrometry. 2) Identifying class-specific regulatory motifs among intracellular induced promoters using a combination of molecular and computational techniques. 3) Characterizing the roles in the M. tuberculosis-macrophage interaction of selected intracellular induced M. tuberculosis genes. Assays will include bacterial survival, replication and trafficking in macrophages. 4) Assessing the role of cAMP signaling in M. tuberculosis within macrophages by defining the distribution and expression among mycobacteria of genes encoding novel cyclase and cyclic NMP binding proteins; estimating the minimum number of cAMP-responsive proteins using 2D gels; and determining the effects of a novel adenylate cyclase gene knock-out on M. tuberculosis interaction with macrophages using tissue culture, microscopy, and 2D gel analyses. This work will contribute to our understanding of the factors needed for the establishment of tuberculosis infection and disease and will identify potential targets for tuberculosis vaccines, therapeutics, and diagnostic purposes.

Grant: 1R01AI045707-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: ORME, IAN M
Title: STRATEGIES FOR TUBERCULOSIS VACCINE DEVELOPMENT AND SCRE
Institution: COLORADO STATE UNIVERSITY-FORT FORT COLLINS, CO
COLLINS
Project Period: 2000/05/01-2005/04/30

The purpose of this application in response to PA-97-101, "Basic mechanisms of vaccine efficacy", is to conduct basis research into mechanisms of vaccine-induced immunity in the lungs, using a highly pertinent and relevant tuberculosis infection model. An underlying motive is the plethora of new TB vaccine candidates currently being screened in mouse and guinea pig models, in which the Mycobacteria Research Laboratories [MRL] at Colorado State University are playing a central role. As the number of vaccine candidates tested has gradually increased, it has become increasingly apparent that fundamental information regarding vaccine strategies against this serious bacterial respiratory pathogen, particularly as it pertains to expression of immunity in the lungs, is almost completely lacking. Accordingly, this application proposes to address four major areas of concern, which in each case has the potential to derive important new information which may improve and broaden our overall approach to tuberculosis vaccination. These areas are [1] development of a widely applicable approach to identification of potential protein antigens, and specific application of this approach to the identification of key target antigens of Mycobacterium tuberculosis, an approach that will draw on our considerable experience in bacterial cultivation, antigen extraction, and proteomic analysis; [2] a further exploration of the potential for cytokine enhancement of vaccination, based on our recent success in this area; [3] work to determine the basis of down-regulation in vaccination, specifically in terms of interference with the BCG vaccine; and [4] the nature and antigenic targets of the CD8 T cell response in the lungs and the potential for CD8-directed vaccination strategies. These studies will exploit our extensive state of the art Level III biohazard facilities, our expertise in bulk cultivation and antigen purification, our new access to sophisticated cell sorting technology, and our demonstrated experience in using and developing the mouse low dose aerosol tuberculosis infection model.

Grant: 1R01AI045715-01A1
Program Director: KLEIN, DAVID L
Principal Investigator: WEISS, ALISON A
Title: HUMAN SERUM AND PROTECTION AGAINST BORDETELLA PERTUSSIS
Institution: UNIVERSITY OF CINCINNATI CINCINNATI, OH
Project Period: 2000/07/01-2005/06/30

DESCRIPTION (Adapted from the Applicant's Abstract): The ability of whole cell and acellular pertussis vaccines to protect against severe pertussis has been clearly demonstrated, however a large proportion of vaccinated individuals exposed to *B. pertussis* become infected, and many develop a cough that lasts for weeks. The nature of the protective response is not well understood, and without this knowledge it will not be possible to improve the pertussis vaccine. The investigators propose to use functional assays to assess immunity using serum from humans with various types of exposure (infected individuals, vaccinated individuals, and negative controls). They will examine complement killing, opsonization, and phagocytosis of *Bordetella pertussis*, and pertussis toxin neutralization. Specific AIM 1-Antibodies that mediate complement killing. The BrkA protein of *B. pertussis* confers resistance to killing by complement, however some individuals produce an antibody response that is able to overcome this resistance mechanism. They will identify the targets of the bactericidal antibodies. Specific AIM 2-Antibodies that mediate opsonization and phagocytosis. The ability of human antibodies to promote opsonization and phagocytosis by macrophages, monocytes, and neutrophils will be evaluated using bacteria labeled with green fluorescent protein. They will examine the ability of virulence factors such as adenylate cyclase toxin and pertussis toxin to interfere with this process, and they will identify antibodies that promote opsonization. Specific AIM 3-Neutralization of pertussis toxin. They will evaluate the ability of human antibodies to neutralize pertussis toxin toxicity to human neutrophils. In addition, they will assess if people are more likely to generate neutralizing antibodies if immunized with genetically toxoided toxin, chemically toxoided toxin, or by natural disease.

Grant: 1R01AI045724-01A1
Program Director: SAVARESE, BARBARA M.
Principal Investigator: ROMPALO, ANNE M MD OTHER AREAS
Title: IMPACT OF SOCIAL NETWORKS ON SYPHILIS TRANSMISSION
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 2000/04/01-2005/03/31

DESCRIPTION (Adapted from the Applicant's Abstract): The investigators propose to examine the role of social context and social influence on syphilis transmission in Baltimore. Currently, Baltimore has the Nation's highest rates for newly acquired primary and secondary syphilis. The goals of this study are, first, to examine the social context of syphilis risk through the assessment of social and sexual network characteristics. In collaboration with the Baltimore City Health Department (BCHD), the investigators will recruit between 400 and 1200 patients who present to the BCHD Sexually transmitted Diseases (STD) clinics for evaluation and treatment of primary and/or secondary stage syphilis. The investigators will collect specimens from these patients' syphilis lesions for restriction fragment length polymorphism (RFLP) analysis, and conduct social and sexual network interviews with these syphilis patients and their social/sexual network members. Using Geographic Information System (GIS), the investigators will map the social and sexual networks. This will allow us to track and compare possible syphilis transmission through both network types and to examine social structural factors, especially drug use, which may be associated with disease transmission and risk behaviors. Social context data will be confirmed by biologically-based strain typing. The investigators propose to apply the RFLP technique in collaboration with Dr. Sheila Lukehart at the University of Washington to determine the prevalence of and factors associated with genetic clustering of syphilis in Baltimore over time. The investigators will determine if different RFLP profiles exist in Baltimore, use GIS to plot their spatial distribution and evaluate the relationship of social networks to clusters of infections. This will be the first time that a biological marker of transmission will serve to validate epidemiological defined transmission groups and thus improve our ability to delineate the sexual, social and personal network characteristics associated with syphilis transmission. The investigators are currently funded to examine the role of social context on gonorrhea transmission. As a second goal of this study will be to compare the social context of syphilis risk to that of gonorrhea risk and to determine and compare the role of drug use and other social factors in the social context of both sexually transmitted diseases. Thus, the investigators propose to compare the efficacy of detecting early infectious (primary and secondary stage) syphilis cases by interviewing and screening social network members of early syphilis index cases compared to that of standard sexual partner notification techniques. The proposed project seeks five years of support to map, analyze and compare syphilis cases within social and sexual networks. Data collected in this proposal data may be applied to modify current methods of syphilis contact tracing and develop more effective future preventive and intervention strategies.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01AI045725-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: GILLIS, THOMAS P PHD VET
MEDICINE:MICROBIOLOGY
Title: DEVELOP AND EVALUATE NEW LEPROSY AND TB VACCINES
Institution: NATIONAL HANSEN'S DISEASE PROGRAM BATON ROUGE, LA
Project Period: 2000/07/01-2004/06/30

DESCRIPTION: (Adapted from Applicant's Abstract) Project Goals: The hypothesis to be tested is that vaccination against *M. leprae* with DNA vaccines and TB vaccines will (1) elicit a measurable immune response to appropriate antigens of *M. leprae* and (2) induce significant protection against a *M. leprae* challenge in the mouse footpad model. The DNA vaccines will also be tested for their ability to protect mice against challenge with virulent *M. tuberculosis*. Research Plan: The experimental design is to create and evaluate DNA vaccines expressing mycobacterial protein antigens known to elicit immune responses in humans infected with either *M. leprae* (ML) or *M. tuberculosis* (MT) and evaluate four newly created MT vaccines against leprosy. Specific-objectives are (1) prepare plasmid constructs (DNA vaccines) with genes expressing mycobacterial protein antigens and test immune response elicited by DNA vaccines delivered either by the intramuscular (IM) or intradermal (ID) route, (2) evaluate the efficacy of 4 newly developed TB vaccines for their protective efficacy against ML challenge in mice. The TB vaccines are: 1) leucine auxotroph of BCG, 2) leucine auxotroph of MT, 3) MT culture filtrate in adjuvant prepared from the nontoxic derivative of lipid A from *Salmonella minnesota* (MPL, [plus IL-2]) and 4) pooled MT genomic vaccine, (3) test protective efficacy, potency and immunotherapeutic potential of DNA vaccines in the mouse foot pad model for leprosy and (4) test the protective efficacy and potency of ML DNA vaccine against MT challenge. Mice will be immunized with test vaccines and immune responses will be monitored by lymphoproliferative, cytokine and systemic antibody responses. ML challenge will be in the foot pad-and MT challenges will be by aerosol and intravenous injection. Quantitative bacteriology will be performed on both protection models and histopathology of the immunization sites will be performed. Potency and immunotherapy of vaccines will be tested in the leprosy footpad model by monitoring induction of long-lived protection and effects of vaccines following ML infection, respectively. Significance: Leprosy is a significant public health problem globally with new case estimates of over one-half million yearly and a worldwide prevalence of approximately 1.26 million. Elimination of leprosy will most certainly require more than chemotherapy. A new vaccine for leprosy would provide the greatest potential impact for controlling and possibly eliminating the disease. New vaccines for TB and leprosy are being created using new technologies such as DNA vaccination and auxotrophic mutants of new and existing bacteria. This study proposes to create and evaluate new vaccines for leprosy and tuberculosis and determine their cross-protective efficacy for both diseases.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01AI045737-01A1
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: BASEMAN, JOEL B PHD
MICROBIOLOGY:MICROBIO
OGY-UNSPEC
Title: MYCOPLASMA PNEUMONIAE - AIRWAY INTERPLAY
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR SAN SAN ANTONIO, TX
ANT
Project Period: 2000/04/01-2005/03/31

The epithelial lining of air spaces of the human respiratory system provides a crucial barrier between inspired gas and pulmonary blood flow. By virtue of its large surface area, delicate membranes and anatomic location, the epithelial barrier is vulnerable to damage by inhaled toxic substances and attack by infectious agents. The cell wall-less bacterium, *Mycoplasma pneumoniae* (whose genome is completely sequenced), is a common bacterial pathogen of the human respiratory tract. It causes a range of acute and chronic illnesses, including tracheobronchitis, pneumonia and other airway pathologies as well as extrapulmonary manifestations, such as joint, CNS and cardiovascular involvement. This microorganism exhibits a flask-like appearance and adheres to respiratory cell surfaces via a unique tip-like terminal organelle. In general, bacterial adherence is a complex process involving multiple interactions and molecular cross-talk between the microbe and target cell. We have been investigating mechanisms of cytoadherence of *M. pneumoniae* and other mycoplasmas, and our recent studies indicate that various components in the airway microenvironment contribute to mycoplasma-respiratory cell interactions and subsequent tissue colonization. Delineating the role of such environmental components in the infectious process of mycoplasmas, particularly *M. pneumoniae*, is a major objective of this proposal. We will focus on understanding how fibronectin (FN) and surfactant protein A (SP-A) influence the mycoplasma-airway cell interplay, as these host proteins exist abundantly in the respiratory tract. These proteins play several roles including i) modulating alveolar macrophages in enhanced phagocytosis of invading bacteria; and ii) assisting bacteria (particularly FN) in binding to non-phagocytic cells. We have discovered the presence of FN- and SP-A binding proteins in *M. pneumoniae*, and we intend to clarify the role of these proteins as mediators of mycoplasma- airway cell interactions. Thus, the proposed study is expected to provide new insights concerning mycoplasma parasitic mechanisms which, together with the existing knowledge base concerning mycoplasma cytoadherence, will broaden our overall understanding of mycoplasma pathogenesis and virulence determinants. Ultimately, these studies should identify new therapeutic strategies to control mycoplasma infections in humans.

Grant: 1R01AI045738-01A1
Program Director: MILLER, MARISSA A.
Principal Investigator: TOMASZ, ALEXANDER PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: S.AUREUS CELL WALLS AND DRUG RESISTANCE IN MRSA AND VRSA
Institution: ROCKEFELLER UNIVERSITY NEW YORK, NY
Project Period: 2000/03/01-2005/02/28

DESCRIPTION (Adapted from the Applicant's Abstract): The appearance of vancomycin-resistant strains among clinical isolates of methicillin-resistant (and multiresistant) *Staphylococcus aureus* places a special urgency on efforts to better understand the mechanism of beta-lactam and glycopeptide resistance in these pathogens. The Research Program will have four main foci. (A) A major new development, namely, the successful "activation" of the silent *mecA* homologue of *Staphylococcus sciuri* and its introduction into *S. aureus*, will be followed up by a combination of genetic and biochemical experiments. (B) and (C): New data indicate that the mechanisms of resistance to both methicillin and vancomycin involve a complex stress response in which numerous genetic determinants participate and which is induced by addition of the antibiotic to the growth medium. The drastic alteration in cell wall composition that accompanies expression of resistance will be followed by high-resolution biochemical techniques. The complete genome of the highly and homogeneously methicillin-resistant *S. aureus* (MRSA) strain COL is currently being sequenced at The Institute for Genomic Research (TIGR). With the genome sequence in hand, the plan is to construct a microarray system to explore gene networks operating in the expression of resistance to methicillin and vancomycin. The microarrays will be used to initially define a global transcription profile of COL. This profile will then serve as a basis for comparison in experiments designed to identify changes in gene expression that occur upon the addition of antibiotics or under the influence of specific mutations affecting resistance. Part (D) will concentrate on attempts to identify unique genetic determinants of vancomycin resistance in mutants of COL and in clinical isolates by a combination of transposon mutagenesis and the microarray technology. The project should provide new insights into the mechanisms of beta-lactam and glycopeptide resistance, hitherto unknown genetic and biochemical features of staphylococcal cell wall metabolism, and it may also contribute to the identification of new antibacterial targets.

Grant: 1R01AI045740-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: HANNA, PHILIP C PHD
Title: EARLY ESTABLISHMENT STAGES OF ANTHRAX INFECTION
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2000/09/15-2005/08/31

This proposal investigates the first hours of anthrax infections; in vivo germination of the *Bacillus anthracis* endospore, macrophage survival, growth and escape of the vegetative bacilli. Outside the host, endospores remain metabolically-dormant, preserving virulence even when exposed to harsh environmental conditions. Endospores are the anthrax contagion, entering the body where they are phagocytosed by regional macrophages. Endospores "sense" the new locale, germinate and outgrow to a vegetative state. Our preliminary data defined discrete mutants blocked at each of these steps. After escape, massive bacteremia, toxemia and death ensues. Our data also indicate that anthrax endospores have unique in vivo sensory and signaling mechanisms for triggering germination. Germination occurs rapidly in cultured macrophages. Non-pathogenic *Bacillus* sp. endospores show no increased germination in macrophages. A *B. anthracis* transposon-mediated mutagenesis system allowed selection of individual endospore mutants incapable of germination in macrophages but fully capable of germination and outgrowth in bacterial media. Several unique classes of mutants were characterized. One such loci, named gerP (germination Plasmid), is located on the virulence (toxin) plasmid pXO1. Thus mutations in gerP eliminate host-specific germination but not general germination responses. The aims of this proposal are to: a. define and characterize the germination genes of *B. anthracis* and host chemical signals to determine their roles in the host- specific germination response; b. determine defined intracellular events and bacterial genes used by the vegetative bacilli allowing for survival and escape from the macrophage, and; c. understand the relevance of *B. anthracis* host-specific germination systems and early intracellular events in terms of pathogenesis in the murine model. Knowledge of these critical "establishment" stages of anthrax may provide targets for early intervention after exposure to anthrax endospores. Understanding this rapid and dramatic switch, from absolute metabolic dormancy of the endospore to growing virulent bacilli allows anthrax to be exploited as an effectual model for examining the earliest stages of bacterial infectious cycles.

Grant: 1R01AI045746-01A1
Program Director: LANG, DENNIS R
Principal Investigator: CAMILLI, ANDREW BS
Title: STUDY OF VIBRIO INTESTINAL PHYSIOLOGY AND PATHOGENICITY
Institution: TUFTS UNIVERSITY BOSTON BOSTON, MA
Project Period: 2000/03/01-2005/02/28

Vibrio cholerae is a facultative pathogen and is the causative agent of the severe secretory diarrheal disease cholera. Virtually all cholera at present is caused by El Tor biotype strains which differ biochemically and phenotypically from strains of the better-characterized classical biotype. The goal of the proposed work is to understand the pathogenicity and physiology of El Tor *V. cholerae* during intestinal infection. Laboratory manipulation of virulence factor expression in El Tor strains is difficult, and this has hampered their study. Moreover, knowledge of which genes are expressed by *V. cholerae* during infection is rudimentary, and virtually nothing is known about the patterns of expression of such genes within the gastrointestinal tract. Advanced genetic approaches will be used to identify and analyze El Tor infection-induced genes within the suckling mouse model of cholera, and during colonization of a natural plankton host. The regulation and spatiotemporal patterns of expression of genes in the ToxR regulon, and newly identified virulence genes, will be determined in vivo using an enhanced recombinase-based in vivo expression technology (RIVET). Factors which are non-essential for in vitro growth but are essential for colonization of the small bowel of suckling mice, will be comprehensively identified using a modified Signature-Tagged Mutagenesis (STM) procedure. As a complementary approach, RIVET will be used to identify genes induced transcriptionally during infection, some of which may be essential for in vitro growth. These methods will also be used to identify and characterize *V. cholerae* genes important for colonizing the plankton host *Anabaena variabilis*. The importance of select genes for colonization of each host will be determined by constructing specific gene mutations followed by colonization studies. The broad specificity of RIVET, combined with the focused specificity of STM, will allow identification of an unprecedented number and variety of genes that play roles in the physiology and virulence of *V. cholerae* in these animal and environmental host systems. These studies will not only establish a basis for understanding the dynamics of virulence gene expression during infection of an intact host, but will aid in the development of new cholera vaccines and suggest new approaches for the prevention of the dissemination of this lethal organism.

Grant: 1R01AI045820-01A1
Program Director: MILLER, MARISSA A.
Principal Investigator: SOKURENKO, EVGENI V MD
Title: PATHOGENIC ADAPTATION OF MICROBIAL ADHESINS
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2000/05/01-2005/04/30

Description (adapted from the applicant's abstract): The long-term goal of the application is to understand the role of enterobacterial adhesins in the colonization of extra-intestinal habitats. The proposed study is designed to clarify the molecular mechanism by which mannose-sensitive type 1 fimbriae of *Escherichia coli* mediate development of urinary tract infection. Receptor specificity of the fimbrial adhesive protein, FimH, will be characterized based on its interaction with defined saccharide receptors in soluble and immobilized forms. The relationship between the type 1 fimbrial phenotypes and the primary structure of FimH adhesins will be determined by site-directed mutagenesis of the polymorphic and conserved amino acid residues of FimH. The molecular basis of the different binding properties of FimH variants will be defined based on the functional characteristics of fimbriae-incorporated and purified forms of FimH and by using rabbit antibodies against different FimH regions. The role of the different FimH variants in colonization of vaginal introitus with *E. coli* will be investigated by defining the type 1 fimbrial phenotype of the vaginal isolates and, also, of the matching fecal and urinary clones. Ability of the type 1 fimbriae to mediate *E. coli* adhesion to human vaginal epithelial cells and secretion compounds will be tested. The comparative study of FimH variants proposed will contribute to general understanding of the adaptive evolution of enterobacterial pathogens within extra-intestinal compartments of the human organism. Such information may be useful in optimizing methods to prevent dissemination of pathogens from the human gut.

Grant: 1R01AI045928-01A1
Program Director: LANG, DENNIS R
Principal Investigator: BLANKE, STEVEN R PHD BIOCHEMISTRY
Title: MOLECULAR MECHANISMS OF THE H. PYLORI VACUOLATING TOXIN
Institution: UNIVERSITY OF HOUSTON HOUSTON, TX
Project Period: 2000/03/15-2005/02/28

Helicobacter pylori is an etiologic agent of a number of gastric disorders in humans, including peptic ulceration and gastric adenocarcinomas. Early efforts to understand *H. pylori* pathogenesis revealed the presence of a secreted bacterial toxin (VacA) which mounting evidence indicates is an important virulence factor. VacA causes massive degenerative vacuolation of mammalian cells which has been implicated in the gastric epithelial erosion preceding the onset of peptic ulcer disease. The broad objective of this research program is to elucidate the fundamental mechanisms by which VacA mediates cellular cytotoxicity. Long-term milestones include identification of the biochemical activity and intracellular targets of VacA. Experiments in this proposal are designed to test the hypothesis that VacA mediates cellular cytotoxicity as an AB toxin. The model of VacA as an AB toxin is important because it suggests specific hypotheses directly relevant to the fundamental mechanism by which VacA enters host cells and induces vacuolation. The AB family of toxins share a number of distinct properties, and includes prominent members such as cholera, diphtheria, tetanus, and anthrax toxins. The specific aims in this proposal are designed to elucidate the VacA molecular structure and begin to identify structure-function relationships of the toxins. The specific aims are: [1] To identify cellular mechanisms of VacA cytotoxicity. To achieve these goals, novel VacA fusion proteins will be genetically constructed to determine if VacA is trafficked to the cytosol. In addition, radiolabeled VacA will be analyzed for intracellular proteolytic processing. [2] To identify VacA structural characteristics important to the toxin's cellular activities. To achieve these goals, mutant forms of VacA and novel fusion proteins will be genetically constructed to test hypotheses about VacA structure-function relationships and biochemical activities. Because it is estimated that the prevalence of *H. pylori* infection in developed countries is 20-50%, and 70-90% in developing countries, the importance of developing efficacious vaccines, chemotherapeutics, and diagnostics of *H. pylori* cannot be overstated. These studies will not only contribute to our understanding of the fundamental mechanisms of VacA-mediated cytotoxicity, but may reveal novel strategies for using attenuated VacA, or fragments of VacA as components in new protective vaccines.

Grant: 1R01AI046097-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: ERNST, JOEL D
Title: M TUBERCULOSIS EVASION OF IMMUNE EFFECTOR MECHANISMS
Institution: UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA
Project Period: 2000/06/01-2004/05/31

DESCRIPTION: (Adapted from Applicant's Abstract) Mycobacterium tuberculosis is one of the most successful human pathogens known. To achieve such success, M.tuberculosis must avoid destruction by the immune system. Nevertheless, its mechanisms of evading the immune system are not well understood. The PI tested the hypothesis that M.tuberculosis evades immune effector function by inhibiting the action of interferon gamma, a crucial mediator of innate immunity. The investigators found that M.tuberculosis infection of human macrophages blocks transcriptional activation of interferon gamma responsive genes, and that it does so by disrupting the interaction of the transcription factor, STAT 1, with the transcriptional coactivators CBP and p300. By blocking interferon gamma transcriptional responses, M.tuberculosis can inhibit activation of macrophages and can survive despite development of a cellular immune response. In this application experiments are proposed to identify the mechanism by which M.tuberculosis disrupts the STAT1-CBP interaction, and to determine whether overcoming those mechanisms allows human macrophages to respond to interferon gamma by killing M.tuberculosis. The effects of live M.tuberculosis can be replicated by gamma-irradiated bacteria, as well as by a crude cell wall fraction, but not by LAM. We propose to identify the specific components of the M.tuberculosis cell wall that initiates inhibition of interferon gamma responses, and to characterize macrophage responses to that component. The investigators have also discovered that, in response to M.tuberculosis, macrophages synthesize and release a soluble protein (termed SINGR) that causes inhibition of interferon gamma responses in naive, uninfected cells. SINGR activity is not attributable to known cytokines, such as IL-4, IL-6, IL-10, or TGF-b. The investigators propose to identify SINGR by purification or expression cloning, prepare antibodies that neutralize its activity, and determine whether SINGR is an obligate intermediate molecule in the inhibition of interferon gamma responses by M.tuberculosis. It is hoped that the results of these studies will provide valuable insight into the interaction of M.tuberculosis with the human immune system, particularly the ability of this pathogen to persist in the face of a seemingly appropriate immune response.

Grant: 1R01AI046226-01A1
Program Director: LAUGHON, BARBARA E.
Principal Investigator: CANGELOSI, GERARD A PHD
Title: MULTIDRUG RESISTANT MORPHOTYPIC VARIANTS OF M. AVIUM
Institution: SEATTLE BIOMEDICAL RESEARCH SEATTLE, WA
INSTITUTE
Project Period: 2000/02/01-2004/01/31

Mycobacterium avium is responsible for serious opportunistic infections of AIDS patients and other susceptible individuals. M. avium isolates segregate into transparent and opaque colony variants with different pathogenicity and drug susceptibility characteristics. We have identified a new type of phenotypic variation, in which opaque and transparent variants segregate into red- and white-staining subvariants on agar media containing the lipoprotein stain Congo red. White segregants are significantly more resistant to multiple antibiotics than are red segregants. Opaque-transparent variation was previously believed to be the overriding determinant of drug susceptibility in M. avium, but our observations suggest that red-white variation has a greater impact on susceptibility to some drugs. Using a novel screening approach, we found a genetic polymorphism that co-segregates with the white phenotype. This polymorphism which consists of an open reading frame (ORF1) interrupted by a novel insertion element (IS999), is the first genetic marker of multi-drug resistance to be identified in M. Avium. We propose to 1) determine the role of the ORF::IS999 fusion in red-white variation, 3) characterize the red and white phenotypes in vitro, and 4) determine the impact of red-white variation on disease progression and treatment outcomes in virulence models. Red-white variation is common among clinical isolates of M. avium and a potentially important factor in treatment failure. Understanding of how it occurs could lead to better ways to diagnose and treat multi-drug resistant mycobacterial infections.

Grant: 1R01AI046428-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: CLARK-CURTISS, JOSEPHINE E PHD MICROBIOLOGY, OTS
Title: GENE EXPRESSION IN MYCOBACTERIUM TUBERCULOSIS
Institution: WASHINGTON UNIVERSITY ST. LOUIS, MO
Project Period: 2000/02/01-2004/01/31

Tuberculosis, one of the great scourges of humankind, is the leading cause of death worldwide from a single infectious disease. Although the incidence of TB has again begun to decline in the U.S., TB remains a significant health problem in this country, most frequently affecting the elderly, the homeless, individuals with AIDS and immigrants from nations where TB is endemic. Although the causative agent of TB, *Mycobacterium tuberculosis*, was identified a century ago, knowledge about fundamental physiological capabilities, the genetics and the mechanisms of pathogenicity of *M. tuberculosis* is only now beginning to emerge. We have identified several genes which are expressed by *M. tuberculosis* H37Rv when the bacilli are growing in human macrophages in culture, but which are not expressed by the bacilli when they are growing in laboratory broth culture. We hypothesize that these genes and their products may be important in the survival and growth of *M. tuberculosis* in macrophages and may contribute to the pathogenicity of the tubercle bacilli. We propose to (1) determine the contribution of specific macrophage-expressed gene products to survival and growth of *M. tuberculosis* in macrophages by molecular and genetic characterization of genes on a cosmid that appear to be coordinately expressed, a putative response regulator gene, and *mceD*, a gene that has been shown to enhance survival of *E. coli* in cultured macrophages; and (2) to identify and characterize genes and gene products of *M. tuberculosis* that are expressed at early times after phagocytosis, at late times, and throughout growth in human macrophages in culture.

Grant: 1R01AI046433-01
Program Director: RUBIN, FRAN A.
Principal Investigator: CAPARON, MICHAEL G PHD MICROBIOLOGY, OTS
Title: PROTEASE AND ROP GENES IN S PYOGENES VIRULENCE
Institution: WASHINGTON UNIVERSITY ST. LOUIS, MO
Project Period: 2000/01/15-2004/12/31

The concern over the possible reemergence of severe invasive diseases caused by *Streptococcus pyogenes* (group A streptococcus) has focused attention on the role of its secreted cysteine protease in pathogenesis. Unfortunately and despite extensive application of modern genetic technology, various studies have both supported and refuted an important role for the protease in promoting disease. Thus, the role of the cysteine protease in pathogenesis remains unclear. It is likely that much of the controversy has arisen because the biogenesis of the cysteine protease is only poorly understood. However, it is clear that this process is highly regulated, both at the level of transcription and at the level of processing the secreted inactive pro-protein to the active form. My lab has begun to address this issue and developed a novel transposon to identify three loci required for expression of proteolytic activity. Designated as Rop loci (regulation of protease), *ropB* is an activator of transcription of the gene which encodes the protease and is a member of the emerging Rgg-like family of regulators of which very little is currently known. In contrast, *ropA* contributes to secretion and processing of the protease and encodes a homologue of Trigger Factor, a peptidyl-prolyl isomerase and putative chaperone, which is highly conserved in most bacterial species, but of unknown function. Preliminary studies have shown that RopA acts both to assist in targeting the protease to the secretory pathway and in promoting the ability of the proprotein to establish an active conformation upon secretion. The Specific Aims will address 1.) the function of *ropC*, including whether it is involved in transcription, secretion or processing of protease; 2.) Further characterization of RopB, focusing on its possible interactions with the multiple promoters for the gene which encodes the protease; 3.) an in-depth analysis of the role of RopA in secretion and folding of the protease precursor; and 4.) An examination of the role of the Rop loci in virulence in a murine model of streptococcal infection. The proposed studies will utilize recently developed state-of-the-art methodologies for analysis in *S. pyogenes* and the further characterization of these genes will be important for understanding the function of the protease through understanding when and where it is expressed during infection.

Grant: 1R01AI046454-01
Program Director: LANG, DENNIS R
Principal Investigator: LEONG, JOHN M MD
Title: HOST CELL SIGNALING BY EHEC INTIMIN PROTEIN
Institution: UNIV OF MASSACHUSETTS MED SCH WORCESTER, MA
WORCESTER
Project Period: 2000/02/01-2005/01/31

Enterohemorrhagic *E. coli* (EHEC) has emerged as an important agent of diarrheal disease and the leading cause of pediatric renal failure in the U.S. Intimate attachment to host cells is an essential step during intestinal colonization by EHEC. After initial host cell attachment, the bacterium injects into the host cell a number of molecules that trigger signaling pathways and result in the disruption of the eukaryotic cytoskeleton. Among the injected proteins is Tir, a protein that becomes localized in the host cell membrane and acts as a receptor for the bacterial outer membrane protein intimin. Intimin, encoded by the *eae* gene, is required for the formation of a highly organized for the formation of a highly organized cytoskeletal structure containing filamentous actin directly beneath the bound bacterium that lifts the bacterium above the plane of the host cell membrane on a "pedestal". Deletion mutants of *eae*, which cannot induce the formation of this pedestal, are deficient for intestinal colonization. Thus, we postulate that Tir-intimin interaction is an essential early event in the development of disease caused by EHEC. We have identified regions of intimin and Tir that interact with each other, and have shown that the Tir-binding region of intimin is sufficient to induce actin condensation after pre-infection of host cells with *E. coli*. A detailed understanding of Tir-intimin binding, as well as of the molecular signals immediately downstream of this interaction, are required to gain insight into how EHEC colonizes the intestine and promotes damage. Thus, the following questions will be addressed: 1. What is the topological map of Tir in the eukaryotic membrane? 2. Is Tir binding by intimin sufficient to trigger actin condensation on preinfected cells? Latex beads that artificially bind TIR will be tested for the ability to induce actin condensation on preinfected eukaryotic cells. 3. How does intimin and Tir recognize each other? Genetic and biochemical approaches, including crystallographic studies, will be pursued to understand the molecular basis for this interaction. 4. Is Tir-intimin interaction essential to promote intestinal colonization? Point mutations in *eae* and *tir* that disrupt or restore Tir-intimin binding will be tested for their effect on colonization in an animal model for EHEC infection. 5. What mammalian cell factors interact with the cytoplasmic region(s) of Tir? Mammalian cell factors that directly receive from Tir the biochemical signal for actin filament formation will be identified. The proposed experiments may provide novel targets for therapeutic intervention during EHEC infection, as well as provide insight into the general cellular mechanisms by which actin assembly controlled.

Grant: 1R01AI046464-01A1
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: GRANOFF, DAN M MD
Title: CONSERVED NEISSERIA PROTEINS AS VACCINE CANDIDATES
Institution: CHILDREN'S HOSPITAL & RES CTR AT OAKLAND, CA
OAKLAND
Project Period: 2000/07/05-2003/06/30

DESCRIPTION: (Adapted from Applicant's Abstract) The long-term objective of this study is to increase our understanding of the use of conserved membrane proteins as components of a vaccine for prevention of *Neisseria meningitidis* serogroup B (MenB) disease. MenB is a major cause of meningitis and sepsis. Although serum bactericidal antibodies confer protection, to date, conventional approaches to develop a vaccine have been largely unsuccessful. Polysaccharide-based MenB vaccines risk eliciting autoantibodies to host polysialic acid, while the ability of most non-capsular antigens to elicit broad-based immunity is limited by antigenic diversity. We propose to investigate the vaccine potential of three recently discovered conserved Neisserial membrane proteins, designated Neisserial surface proteins (Nsp) A, B, and C. As backup candidates, NspD and NspE are also available. NspA was discovered with a monoclonal antibody, while the other four proteins represent new vaccine candidates that were discovered from analysis of genomic data. All five proteins are highly conserved across pathogenic *Neisseria*, have epitopes on the surface of the bacteria that are accessible to antibody, and elicit complement-mediated bactericidal antibodies in mice or rabbits. Thus, each of these proteins deserves further investigation as candidate antigens for inclusion in a MenB vaccine. In Aim 1, we will investigate the immunogenicity of each of the recombinant proteins in mice and guinea pigs. Should the recombinant molecules fail to elicit high titers of antibodies that are functionally active against the bacteria, we will attempt to reconstitute conformational epitopes with the use of detergents or liposomes, and explore the use of novel adjuvants suitable for human use. In Aim 2, we will prepare monoclonal antibodies (Mabs) that react with epitopes on the Ns proteins that are important in eliciting protective antibodies. These Mabs will be used for epitope mapping, and for studies of antibody functional activity. In Aim 3, we also will use the 3Iabs to investigate whether there are strain differences in surface accessibility and expression of the different NS proteins, and correlate any differences found with the respective DNA sequences encoding the proteins, or transcriptional activity of the respective genes. We also will investigate whether surface accessibility of the different Ns proteins varies within a Neisserial strain when propagated in vitro, or in infant rats. In Aim 4, we will test the hypothesis that a vaccine containing more than one Ns protein will elicit broader protective immunity to MenB than a vaccine made from a single protein. These results are directly relevant to evaluating the potential for inclusion or exclusion of each of these novel proteins in a MenB vaccine. The data also may validate the genomic approach for identification of new antigenic targets for vaccine development.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01AI046469-01
Program Director: KLEIN, DAVID L
Principal Investigator: CAMPAGNARI, ANTHONY A PHD TUMOR IMMUNOLOG
Title: ANALYSIS OF M CATARRHLIS RECEPTORS AS VACCINE ANTIGENS
Institution: STATE UNIVERSITY OF NEW YORK AT BUFFALO, NY
BUFFALO
Project Period: 2000/02/01-2004/01/31

Moraxella catarrhalis, a human mucosal pathogen, is a prominent cause of otitis media in young children and lower respiratory tract infections in adults with C.O.P.D. The significant financial burden on the health care system in this country, has stimulated research studies aimed at identifying possible vaccine components expressed on the bacterial surface. Despite these efforts, a vaccine target remains elusive. Recent studies have focused on the components of the bacterial outer membrane, as these structures would most likely be available for interaction with the host immune response. However, it is clear that very little is known about the host immune response to infections with *M. catarrhalis*. We have reported studies demonstrating that *M. catarrhalis* can obtain iron from human transferrin and lactoferrin for in vitro growth, and the absence of any detectable siderophore specific proteins on the bacterial surface. We hypothesize that these proteins, named OMP B1, which binds human transferrin and LBP, which binds human lactoferrin, are likely similar to the iron-repressible, receptor proteins expressed by various Gram-negative pathogens. Using monoclonal antibodies we have shown that OMB B1 and LBP are conserved on the surface of all *M. catarrhalis* strains tested to date. In addition, we have shown that children recovering from otitis media have IgG antibodies to both proteins in their convalescent sera. We hypothesize that OMP B1 and LBP contain conserved, surface exposed epitopes which are expressed in vivo and are immunogenic in children. We further hypothesize that OMP BL and LBP are expressed in vivo in response to iron-limitation, thus making these proteins potential targets of the human immune response. The two broad goals of this proposal are to determine the specific structural arrangement of OMP B1 and the LBP and to determine if these proteins have potential value as vaccine components by analyzing the human immune response to each protein in children with otitis media. Our hypothesis will be tested by the following specific aims: (1) To develop and characterize monoclonal antibodies (MABs) directed to different epitopes of a specific transferrin receptor and a lactoferrin receptor. (2) To delineate membrane topography of each receptor and define antigenic domains. (3) To analyze and characterize the human antibody response, elicited in vivo, to each specific receptor.

Grant: 1R01AI046473-01
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: CLEGG, STEVEN PHD
Title: BINDING OF KLEBSIELLA FIMBRIAE TO RESPIRATORY TISSUE
Institution: UNIVERSITY OF IOWA IOWA CITY, IA
Project Period: 2000/01/15-2002/12/31

The type 3 fimbrial adhesin (MrkD) of *Klebsiella pneumoniae* mediates binding of the bacteria to specific types of collagen. The receptors for this adhesin molecule are located in the basement membrane region of tissues, and denuded epithelial surfaces are likely to present binding sites. Since *K. pneumoniae* is a frequent cause of respiratory infections of patients with epithelial trauma, it is possible that type 3 fimbriate bacteria colonize the airways in these individuals due to the presence of the MrkD adhesin. In this proposal we describe experiments we describe experiments to determine the role of the MrkD adhesin in facilitating colonization, *in vivo*, of the respiratory tract. A murine model of respiratory infection will be used and the animals will be treated with dilute HCl or hexamethylphosphoramide to cause desquamation of the mucosa to mimic similar histological lesions seen in compromised individuals. An mrkD-positive isolate of *K. pneumoniae*, and derivatives of this strain that do not express the MrkD adhesin, will be used to investigate the role of the adhesin in colonization. Molecular analysis of variant MrkD adhesin proteins will be performed in order to determine the binding domains within these molecules. Chimeric adhesins possessing defined regions of two different molecules exhibiting receptor binding specificities will be constructed. Binding to specific collagen types will be investigated by standard techniques. Site-directed mutagenesis will be used to further delineate the collagen-binding sites of the adhesin. Also, collagen subunits and fragments will be examined in order to localize the receptor sites on whole collagen molecules. This material will be used in several binding assays using purified fimbrial protein as well as fimbriate bacteria. The identification of receptor sites will allow further investigations into the development of synthetic analogs that could inhibit bacterial colonization of susceptible individuals.

Grant: 1R01AI046509-01
Program Director: GOTTLIEB, MICHAEL
Principal Investigator: DETKE, SIEGFRIED PHD
Title: IDENTIFYING TOR'S MODE OF ACTION
Institution: UNIVERSITY OF NORTH DAKOTA GRAND FORKS, ND
Project Period: 2000/02/01-2003/01/31

It has been estimated that millions are infected with Leishmania and tens of millions more with the related Trypanosomes. Perhaps a half of billion people live in areas endemic to these parasites and are at risk. The established anti-Leishmania compounds are not completely efficacious and adverse reactions have been documented. Similar problems occur with the drugs used for the other Trypanosomes. The purine analogs allopurinol and allopurinol riboside, on the other hand, are toxic to these organisms but relatively benign to humans and have demonstrated some success in the treatment of these parasitic infections. TOR, an atypical multi drug resistance factor, elicits resistance to toxic nucleoside as well as to Pentostam, Amphotericin B and a number of other structurally unrelated compounds. The specific aim of this proposal is to determine how TOR exerts its effects. This will be accomplished by determining the fate of the adenosine permease in Leishmania which express more or less TOR than do wild type cells. Western and northern blotting and ribonuclease protection assays will enable us to determine how TOR affects the activity of this reporter permease. The broad, long term objectives are to manipulate through TOR the sensitivity of Leishmania to toxic nucleosides and the other clinically proven anti-Leishmania compounds. It may be possible through TOR to reduce the purine transport capability and starve the parasite of an absolutely essential nutrient.

Grant: 1R01AI046558-01A1
Program Director: MILLER, MARISSA A.
Principal Investigator: SHERERTZ, ROBERT J MD
Title: THE CLOUD ADULT
Institution: WAKE FOREST UNIVERSITY HEALTH SCIENCES WINSTON-SALEM, NC
Project Period: 2000/07/01-2004/06/30

The broad, long-term objective of this work is to obtain a better understanding of a novel mechanism of microorganism transmission. The central hypothesis is that a viral URI (rhinovirus infection) will cause nasal carriers of *Staphylococcus aureus* to have airborne dispersal of *S.aureus* secondary to nasal mucosal swelling with resultant more rapid airflow over wet surfaces. Experiments in specific Aim 1 will define the natural history of rhinovirus-associated airborne dispersal of *S.aureus* and whether airborne dispersal in this setting is further increased by nose breathing, coughing, or sneezing. The relationship between nasal mucosal swelling and rhinovirus infection will be evaluated using CAT scans, rhinomanometry, and acoustic rhinometry. A second important focus will be to determine the relationship between the number of *S.aureus* in the nose and airborne dispersal. Experiments in specific Aim 2 will be concerned with better defining the mechanism of rhinovirus-associated airborne dispersal of *S.aureus*. A topical decongestant will be used to test the hypothesis that reducing rhinovirus-associated mucosal swelling will prevent airborne dispersal. Stopping the medications of *S.aureus* nasally colonized allergic rhinitis patients will be used to evaluate whether just mucosal swelling is necessary or whether it takes mucosal swelling plus the rhinovirus. The quantitative relationship between the number of *S.aureus* in the nose and airborne dispersal will be tested by using tetracycline treatment to increase the number of tetracycline-resistant *S.aureus* in the nose and using topical mupirocin to reduce the number of *S.aureus* in the nose. Specific Aim 3 will determine the prevalence of single nasal *S.aureus* carrier-associated outbreaks, as well as identify individuals who may be studied to identify what properties make such individuals unique. *S.aureus* is the most important cause of nosocomial infections in the United States. *S.aureus* outbreaks are extremely disruptive and cause significant patient morbidity and mortality. Both "cloud babies" and "cloud adults", newborn infants or healthcare workers nasally colonized with *S.aureus* that airborne disperse *S.aureus* associated with a viral URI, have been shown to cause outbreaks. Understanding the "cloud phenomenon" may facilitate *S.aureus* outbreak prevention and possibly the transmission of other pathogens, as well.

Grant: 1R01AI046582-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: KOLATTUKUDY, PAPPACHAN E PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: GENES FOR METHYL-BRANCHED WALL LIPIDS AND TUBERCULOSIS
Institution: OHIO STATE UNIVERSITY COLUMBUS, OH
Project Period: 2000/01/15-2003/12/31

Resurgence of tuberculosis (TB) and other mycobacterial infections associated with AIDS threaten human health world-wide. Emergence of drug resistant TB makes it critical to discover new drug targets. Cell walls of *Mycobacterium tuberculosis* contain a variety of unusual lipids with many types of multiple methyl-branched fatty acids that are unique to pathogenic mycobacteria. These lipids are thought to play important roles in the ability of the pathogens to resist antimicrobial agents and evade the defense reactions of the host. Biosynthesis of these unique lipids containing multiple methyl-branched fatty acids could offer ideal targets for new anti mycobacterial drugs. Genomic sequencing revealed that a remarkably unique feature of the mycobacterial genome is that it contains an unusually large number of genes involved in lipid metabolism. Based on the homology to mycocerosic acid synthase (mas) we have identified two classes of polyketide synthase (pks)-like genes which contain a full complement of active site domains that should be involved in the catalysis of all of the steps required for the synthesis of multimethyl- branched fatty acids: Class 1 mas-like genes mas-like genes (msl1, msl2 and msl3) that are highly homologous to mas and Class 2 (msl4, msl5, msl6 and msl7) which show a lesser degree of homology to mas. These open reading frames (ORFs) most probably encode the more than eight classes of methyl-branched fatty acids found in *M. tuberculosis*. To elucidate the function of these genes and to examine their possible role in the host-pathogen interaction, we propose to pursue the following specific aims: 1) Determine the functions of Class 1 mas-like genes, msl1, msl2 and msl3 of *M. tuberculosis* by characterization of their products expressed in *M. smegmatis*, and by determination of the biochemical and functional consequences of their disruption. 2) Determine the functions of Class 2 (msl4, msl5, msl6 and msl7) mas-like genes in *M. tuberculosis* genome, by characterization of their products expressed in *M. smegmatis*, and by determination of the biochemical and functional consequences of their disruption. 3) Determine whether lack of specific lipids caused by the above gene-disruptions affects host-pathogen interaction and virulence. The results will help identify cell wall lipids critical for pathogenesis that might be suitable targets for new anti-mycobacterial drugs.

Grant: 1R01AI046588-01
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: FRANKLIN, MICHAEL J PHD
Title: GENE EXPRESSION OF P AERUGINOSA DURING BIOFILM DEVELOPME
Institution: MONTANA STATE UNIVERSITY (BOZEMAN) BOZEMAN, MT
Project Period: 2000/03/01-2005/02/28

Bacteria that cause infectious diseases often grow in biofilms where the bacterial cells and their extracellular polymers are attached to biological or non-biological surfaces. Bacteria growing in biofilms demonstrate a variety of phenotypic differences compared to the same strains growing planktonically, including increased resistances to antimicrobials and to phagocytosis. This proposal is designed to elucidate the molecular mechanisms that enable bacteria to grow in biofilms and cause chronic, debilitating infections. Much progress has been made on the mechanisms used by bacteria to adhere to surfaces. However, there is little information about the physiological changes in bacterial that occur following bacterial adhesion and during biofilm development. We developed novel in situ genetic and microscopic methods to characterize the changes in gene expression that mediate these phenotypic changes. Using these novel selections, we isolated nine clones with fusions to genes that demonstrated a five-fold to thirty-fold increase in gene expression, following attachment of the opportunistic pathogen *Pseudomonas aeruginosa* to a surface. The goals of this proposal are designed to: (i) characterize the pathogen *Pseudomonas aeruginosa* to a surface. The goals of this proposal are designed to: (i) characterize the products of these surface-growth induced genes (sgi), (ii) determine the survival advantage imparted by sgis during biofilm development, and (ii) identify other changes in gene expression including gene repression, that occur during biofilm development of *P. aeruginosa*. Information provided by this research will increase our understanding of the physiology of bacteria growing in biofilms. This information will provide important targets for anti-microbials, either through inhibition of sgis required for biofilm development, or through aberrant induction of genes normally repressed during biofilm growth.

Grant: 1R01AI046600-01
Program Director: LANG, DENNIS R
Principal Investigator: AZAM, FAROOQ PHD
Title: VIBRIO CHOLERAEE PLANKTON COLONIZATION
Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA
Project Period: 2000/03/01-2005/02/28

The human pathogen *Vibrio cholerae* is widely distributed along many coastlines including those of the United States. Environmental factors play a major role in determining the distribution of toxigenic *V. cholerae*. For example, the introduction of the seventh pandemic of cholera to Latin America in 1991 has been suggested to have been correlated with marine plankton blooms triggered by a climate change event such as that initiated by El Nino. In a effort to better understand marine vectors and conditions influencing the spread of cholera, we propose to examine the association of a variety of clinical and environmental strains of *V. cholerae* with marine plankton. Marine mesocosm experiments will be performed in which *V. cholerae* will be identified by marking cells with a modified version of the green fluorescent protein gene. growth of *V. cholerae* in the mesocosm will be followed using the frequency of dividing cells technique coupled to an image analysis method. Using selected marine model systems *V. cholerae* plankton colonization will be further examined, and the possible role of chemotaxis in plankton or phytodetritus association will be assessed. We will also isolate and characterize, or obtain mutants deficient in plankton colonization. The genetic defects will be identified, and the relationship, if any, between colonization of plankton and of mammals will be ascertained. Finally, the effect of biological and physiochemical factors on *V. cholerae* growth and distribution in mesocosms will be determined and the possibility of cholera toxin phage production and toxin gene exchange among cells in the marine environment will be explored. By understanding how biological, chemical, and physical factors influence the distribution, abundance and virulence of *V. cholerae*, together with the elucidation of the genetic requirements for its persistence with plankton in coastal ecosystems, it will be possible to better predict when environmental change is likely to present a cholera public health risk.

Grant: 1R01AI046610-01
Program Director: MILLER, MARISSA A.
Principal Investigator: CHAMBERS, HENRY F
Title: PENICILLIN INTERACTIVE PROTEINS OF STAPHYLOCOCCUS AUREUS
Institution: UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA
Project Period: 2000/02/01-2004/01/31

Methicillin-resistant strains of *Staphylococcus aureus* are a major clinical problem. They are multiple drug resistant, but ineffectiveness of penicillins and beta-lactam antibiotics is the real problem, as these are drug of choice to treat staphylococcal infections. The objective of this research is to further knowledge of mechanisms of methicillin resistance. Resistance is determined by several proteins that interact with penicillin. The interactions among these proteins are critical, but poorly understood. Knowledge of these interrelationships may lead to new drug discovery and new and more effective approaches to therapy./ Resistance is mainly due to production a novel low affinity penicillin bind protein, PBP 2a, a well wall synthetic enzyme. PBP 2a seems to substitute for all other PBPs. *mecA*, the gene encoding PBP 2a, is regulated by the same regulatory genes that control production of inducible beta-lactamase. Another type of penicillin interactive protein, a penicillin sensory signal transducer *BlaR1*, signals the cell to express PBP 2a and beta-lactamase, which together mediate all beta-lactam resistance in staphylococci. *BlaR1* appears to be a PBP fused to an intracellular Zn^{++} metalloprotease, and as such may represent a completely new type of transmembrane signaling system. There are three aims. Aim 1. To determine the intracellular pathway by which penicillin binding to *BlaR1* signals induction of beta- lactamase and PBP 2a. The effect of specific mutations in *BlaR1* on signaling will be determined to prove whether or not *BlaR1* is a metalloprotease. Putative consensus motifs of this superfamily of proteins will be targeted. The relationship between *BlaR1* activation and proteolysis of *BlaI*, the repressor of the beta-lactamase regulon, will be defined. Aim 2. To identify PBPs, structural determinants, and other elements that interfere with PBP 2a mediated resistance. Effects of PBP deletion and mutations on PBP 2a mediated resistance will test whether PBP 2a can substitute for other PBPs and where essential functions reside within the molecule. The curious phenomenon of negative selection for expression of PBP 2a that we observed in *mec* naive cells also will be examined. Aim 3. To determine when during the cell cycle PBPs are expressed and where they are localized. An electron microscopic method for immunolocalization of specific myc-targeted PBPs in the cell will be developed. To augment information about where PBPs localize, when they are expressed during the cell cycle will be determined by Northern blotting.

Grant: 1R01AI046611-01A1
Program Director: MILLER, MARISSA A.
Principal Investigator: MCCAFFERTY, DEWEY G PHD
Title: BIO-ORGANIC MECHANISMS OF PEPTIDE ANTIBIOTICS
Institution: UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA
Project Period: 2000/06/01-2005/05/31

Bacterial resistance to antibiotics has seriously limited our capacity to overcome infectious disease. Cases of resistance have emerged in virtually all hospital-acquired pathogen-antimicrobial combinations. Soon our most serious infectious threats will be untreatable given our dwindling arsenal of effective antibiotics. Our long-term research goals are to develop synthetic access to biologically interesting peptide antibiotics, to gain insight into their mechanism/mode of action, and to apply the knowledge gained to the development of alternative antibiotics with improved activity against resistant phenotypes. This proposal describes the total synthesis and mechanistic characterization of Ramoplanin, a novel beta-sheet lipodepsipeptide antibiotic with proven activity against methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium*, and cephalosporin-resistant *Streptococcus pneumoniae*, three important Gram positive opportunistic human pathogens. By an unclear mechanism, Ramoplanin appears to arrest bacterial cell wall development at the level of MurG, a glycosyltransferase involved in an intermediate stage of peptidoglycan biosynthesis. Since MurG activity is essential for proper bacterial cell wall development, it is an attractive target for antibacterial design. Harnessing the clinical antibiotic potential of Ramoplanin critically hinges on gaining synthetic access to its structure and deconvoluting the most intimate details of its mechanism of action. To accomplish this we will synergistically merge total synthesis, mechanistic enzymology and protein biophysics to completely correlate structure to antibiotic function. We plan to synthesize Ramoplanin and related analogues using solid-phase methods, thus providing a general synthetic route to favorably modulate its physiochemical properties. We plan to identify the molecular target of Ramoplanin and determine the interaction energies, specificities, and structure of the inhibitory complex. We will assess the inhibitory effect of Ramoplanin on the MurG reaction and on the mechanistically related peptidoglycan transglycosylation cross-linking reaction that takes place on the outer surface of the bacterial cell membrane. Collectively these studies will provide a clear picture of the mechanism of Ramoplanin inhibition of peptidoglycan biosynthesis and promote the design, synthesis, and biological evaluation of a new generation of antibiotics capable of combating bacterial resistance to antibiotics.

Grant: 1R01AI046613-01
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: INGALLS, ROBIN R. MD
Title: ROLE OF LOS AND ITS RECEPTORS IN GONOCOCCAL PATHOGENESIS
Institution: BOSTON MEDICAL CENTER BOSTON, MA
Project Period: 2000/01/15-2004/12/31

Description (Adapted from the applicant's abstract): *Neisseria gonorrhoeae* is a major cause of sexually transmitted diseases. While this organism primarily infects the lower genital tract, it can ascend to the upper female genital tract, and certain strains are capable of dissemination. Early events in the establishment of infection involve interactions between *N. gonorrhoeae* and cells present in the human genital tract. Here, surface antigens on the gonococcus trigger the local and systemic humoral immune response that results in the release of cytokines, prostaglandins, and other inflammatory mediators. Previous efforts have focused on defining immunologic responses to protein antigens on the surface of *N. gonorrhoeae*. In contrast, little attention has been paid to the pro-inflammatory effects of the endotoxin lipopolysaccharide (LPS) that coats the surface of all Gram-negative bacteria, including the gonococcus. With *Neisseria meningitidis*, as well as most enteric Gram-negative pathogens, it is clear that the acute cytokine response associated with the sepsis syndrome is due, in a large part, to the interaction of LPS with its receptors. For *N. gonorrhoeae*, however, the role of its endotoxin (also known as lipooligosaccharide or LOS) their responsiveness to various strains of gonococci and their LOSs. These in the activation of epithelial cells encountered during mucosal infection of the genital tract are unproven, although its pro-inflammatory activity *in vitro* has been documented. The goal of this proposal is to characterize the role of gonococcal LOS in the interaction between *N. gonorrhoeae* and the epithelial cells found in the female genital tract. First, the PI will characterize three novel epithelial cell lines derived from the female genital tract in terms of cell lines may represent a new *in vitro* model for examining the pathogenesis of gonococcal infections. Second, the PI will make two mutants in the lipid A component of gonococcal LOS. Lipid A has been shown to be responsible for the pro-inflammatory effects of LPS, and loss or modification of lipid A would be expected to impact on the pathogenicity of a Gram-negative bacterium. Finally, the PI will examine the role of epithelial cell receptors for endotoxin in gonococcal invasion and activation, with an emphasis on Toll, a family of receptors recently identified as components of the LPS signaling pathway.

Grant: 1R01AI046645-01
Program Director: KLEIN, DAVID L
Principal Investigator: METLAY, JOSHUA P BA
Title: RISK FACTORS FOR DRUG RESISTANT PNEUMOCOCCAL PNEUMONIA
Institution: UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA
Project Period: 2000/04/01-2004/03/31

DESCRIPTION Adapted from the Applicant's Abstract): The emergence of antimicrobial drug resistance among isolates of *S. pneumoniae* threatens to have a major impact on the management of patients with community-acquired pneumonia (CAP). *S. pneumoniae* is the most common cause of CAP and adequate pneumococcal coverage is at the center of empirical guidelines for the management of this disease. Yet, risk factors for infection with drug-resistant vs. drug-susceptible *S. pneumoniae* are controversial and the clinical impact of in vitro levels of resistance is poorly understood. This study will focus on resistance to penicillin because penicillin and related β -lactam antimicrobials are a major component of current empirical treatment options for patients with CAP. Thus, understanding the risk factors for penicillin resistance can make a major contribution to the empirical management of these patients. The primary aim of this study is to identify risk factors for penicillin drug resistance in patients with bacteremic pneumococcal pneumonia. The primary study hypothesis is that both individual and community risk factors independently predict infection with penicillin resistant *S. pneumoniae* in patients with bacteremic pneumococcal pneumonia. A secondary hypothesis is that the duration of prior antibiotic use is a strong independent predictor of penicillin resistance in these patients. The secondary aims of this study are (1) to develop a prediction rule for penicillin resistance in patients with bacteremic pneumococcal pneumonia and (2) to measure the impact of different levels of penicillin resistance on medical outcomes in these patients. The hypotheses for these secondary aims are that (1) a prediction rule can be developed which accurately categorizes patients into high and low risk groups in order to improve the empirical selection of antimicrobial therapy for patients with CAP and (2) the in vitro level of penicillin resistance, alone, is a poor predictor of medical outcomes, but categorization of the adequacy of antimicrobial drug coverage, based on drug susceptibility profiles and pharmacodynamic drug considerations, will be a strong predictor of medical outcomes. This study is a population-based, case-control study, enrolling all hospitalized patients diagnosed with bacteremic pneumococcal pneumonia within the Pennsylvania Delaware Valley. Risk factors will be identified through patient interview and outpatient and inpatient medical record review. Pneumococcal drug resistance will be categorized at our microbiology laboratory with standardized susceptibility testing of all pneumococcal blood isolates. Finally, a cohort study will be completed within the study design, by examining outcomes for all patients, stratifying by pneumococcal drug susceptibility and adequacy of antimicrobial drug coverage.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01AI046667-01A1
Program Director: KLEIN, DAVID L
Principal Investigator: SCHREIBER, JOHN R
Title: HUMAN T CELL RESPONSE TO PNEUMOCOCCAL CONJUGATE VACCINES
Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH
Project Period: 2000/07/01-2005/06/30

Antibodies against the various pathogenic serotypes of pneumococcal capsular polysaccharides (PnPs) provide protection from infection, but pure PnPs are T cell independent antigens and are poor immunogens in children and elderly. Conjugation of PnPs to a carrier protein improves the PnPs-specific antibody (Ab) response and elicits T cell help. The experimental pneumococcal-protein conjugate vaccine uses purified PnPs-specific B cell precursors or whether it is due to the effect of the serotype of the PnPs-specific B cell precursors or whether it is due to the effect of the serotype of the PnPs on the antigen processing of CRM197 yielding alterations in subsequent T cell help in humans. We will immunize fifty adult volunteers with the experimental 7 valent PnPs- CRM/197 vaccine and measure Ab levels against each serotype as well as CRM/197. We will also determine if donor peripheral CD4+ T cells specific for certain CRM/197 epitopes are associated with better immunogenicity of PnPs. Next, we will examine the frequency of PnPs-specific B cell precursors in each donor and determine if PnPs-specific B cell frequency correlates with PnPs Ab titer. We will also determine if PnPs antibody V region gene usage is different for low responder serotypes. Finally, we will examine whether the serotype of PnPs affects processing and presentation of carrier protein-derived epitopes by human antigen processing cells, yielding differences in subsequent T cell help. These data will significantly add to our understanding of the immune response to conjugate vaccines and may provide information useful to the design of second-generation vaccines.

Grant: 1R01AI046669-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: ALLAND, DAVID E MD CLINICAL MEDICAL SCIENCES, OTHER
Title: POPULATION BASED INVESTIGATIONS OF TUBERCULOSIS
Institution: MONTEFIORE MEDICAL CENTER (BRONX, BRONX, NY NY)
Project Period: 2000/05/01-2005/04/30

DESCRIPTION (adapted from applicant's abstract): The rational design and implementation of the next generation of therapies and vaccines against *Mycobacterium tuberculosis* will require a thorough understanding of the mechanisms of antibiotic resistance and bacterial pathogenesis as they apply to human infections. In this proposal, population-based genetic studies of human specimens will be used to determine the clinical consequences of mutations in genes associated with bacterial antibiotic resistance and virulence. Previous population based genetic studies of *M. tuberculosis* have been limited in scope due to the difficulty and expense of large scale DNA sequencing or DNA chip analysis. The applicants have developed molecular beacon PCR assays which are expected to allow them to rapidly and accurately screen large numbers of samples for specific genetic mutations with the accuracy of a single base pair. They will screen for the presence of larger insertions and deletions in DNA sequence using a modified slot blot cross-hybridization approach. They propose to use novel transducing phage techniques to induce homologous recombination and gene substitution in *M. tuberculosis*. These techniques and more established methods will be used to study clinical *M. tuberculosis* isolates acquired by three large scale epidemiological studies underway in Arkansas, San Francisco, and Orizaba, Mexico. DNA sequence analysis will be combined with classical and molecular epidemiology to study the mutational events that lead to resistance to isoniazid (INH), to determine the distribution of specific mutations in susceptible and resistant isolates, and to discover other mechanisms of isoniazid resistance that develop in clinical strains. Transducing phage assays will be used to uncover and characterize additional resistance mutations. A similar approach will be applied to investigate infectivity and virulence in clinical *M. tuberculosis* strains. The complete genomic sequence differences between the laboratory strain H37Rv, and a highly virulent clinical strain CSU093, have recently become known. Studies of these sequence variations in clinical strains will be used to determine the associations between specific mutations and the phenotypes of infectivity and virulence.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01AI046675-01A1
Program Director: FAIRFIELD, ALEXANDRA
Principal Investigator: CARRUTHERS, VERNON B PHD
Title: MICRONEME FUNCTION IN TOXOPLASMA
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 2000/06/01-2005/05/31

Toxoplasma gondii is a major opportunistic pathogen that causes severe disease (toxoplasmosis) in congenitally infected babies and immunocompromised individuals such as those suffering from AIDS. Because it is an obligate intracellular parasite, T. gondii must invade a host cell to survive and replicate. Furthermore, T. gondii invasion is directly responsible for the pathology of toxoplasmosis, since subsequent intracellular replication and egress destroys the infected host cell. Thus, a better understanding of T. gondii invasion could lead to the development of treatments for toxoplasmosis based on inhibition of invasion. Our recent studies indicate that parasite secretion of organelles called micronemes is essential for T. gondii invasion. Thus, the long-term goal of this proposal is to elucidate the function of micronemal proteins in an effort to identify new potential targets for treating toxoplasmosis. We will begin by focusing on MIC2 because our studies suggest that this micronemal protein likely functions as an important adhesin for T. gondii invasion. Specific Aim I will be to use RNA antisense inhibition to measure the importance of MIC2 for T. gondii invasion of host cells and gliding motility. Specific Aim II will be to use deletion and site-specific mutagenesis to identify the sites on MIC2 that mediate cell adhesion. Specific Aim III will be to identify host cell receptors recognized by MIC2. These studies will directly lead to a better understanding of the molecular mechanisms underlying T. gondii invasion of host cells.

Grant: 1R01AI046682-01A1
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: PESCI, EVERETT C PHD
Title: ROLE OF A NEW SIGNAL MOLECULE IN P. AERUGINOSA VIRULENCE
Institution: EAST CAROLINA UNIVERSITY GREENVILLE, NC
Project Period: 2000/07/01-2005/06/30

Pseudomonas aeruginosa is the most prevalent Gram negative bacteria found in patient with hospital-acquired infections and produces a high mortality rate in both immunocompromised and cystic fibrosis patients. This opportunistic pathogen produces an arsenal of virulence factors, some of which are controlled by the cell density monitoring mechanism as quorum sensing. Quorum sensing involves a signal molecule, the autoinducer, that builds in concentration with bacterial density until a threshold concentration is reached where it binds and activates a transcriptional activator protein. *P. aeruginosa* uses two quorum sensing systems, *las* and *rhl*, to control numerous virulence factors (including LasB elastase) through two primary autoinducers, 3-oxo-C12-HSL and C4-HSL. Recently a third inducer molecule designated as the *Pseudomonas* Quinolone Signal (PQS) was discovered. Preliminary results show that PQS is regulated by the *las* quorum sensing system and that it requires at least Rh1R to inducer *lasB*. To elucidate the PQS synthetic pathway, genes characterize factors controlling PQS expression. To elucidate pathway, genes responsible for anthranilate (a PQS precursor) synthesis will be studied, and a phenotypic, and a phenotypic screen based on *LasB* production will be used to clone other genes responsible for PQS production. To define the role of PQS in *P. aeruginosa* virulence, reporter gene fusions will be used to determine how PQS affects the expression of different virulence genes. Finally, to study PQS expression, or PQS bioassay will be used to monitor its production under various conditions. The long term goal of this proposal is to determine the role of PQS in the pathogenesis of *P. aeruginosa* infections with the hope it will lead to new and effective therapies against *aeruginosa*.

Grant: 1R01AI046966-01A1
Program Director: LAUGHON, BARBARA E.
Principal Investigator: MESHNICK, STEVEN R MD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: YEAST MODELS OF DRUG-RESISTANT PNEUMOCYSTIS CARINII
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2000/04/01-2005/03/31

DESCRIPTION: (Adapted from Applicant's Abstract) Pneumocystis carinii Pneumonia (PCP) is the major life-threatening opportunistic infection associated with AIDS. Recently presented molecular evidence from this group suggests that drug-resistant *P. carinii* is emerging. This could have serious public health implications. The evidence for drug resistance, however, is only indirect, since we are unable to reliably assess in vitro drug sensitivities of *P. carinii* clinical isolates. The goal of this project is to use yeast models to determine whether, and to what extent, specific mutations confer drug resistance. The investigators will focus on two genes - dihydropteroate synthase (DHPS), which is the target for sulfa and sulfones, and cytochrome b, which is the target for atovaquone. Mutations in the *P. carinii* DHPS gene have been found which are associated with sulfa prophylaxis failures. Mutations in the *P. carinii* cytochrome b gene in patients failing atovaquone prophylaxis have been found as well. These mutations might confer resistance since they are similar to mutations that cause resistance to related compounds in other organisms. The aims of the proposed project are to: Engineer clinically observed DHPS mutations into transgenic yeast (DHPS *Saccharomyces cerevisiae* expressing the *P. carinii* sp.f. hominis DHPS). Then the investigators will determine whether and to what extent these mutations confer resistance by assessing the effects of SMX on growth and on the DHPS activity of the purified recombinant protein. Construct yeast clones with mutations in their mitochondrially-encoded cytochrome b genes that are identical or similar to those found in *P. carinii* clinical isolates. The investigators will then determine how atovaquone affects a) the growth of the mutant strains in culture and b) electron transport in mitochondria isolated from these strains. Determine whether other sulfa drugs or hydroxynaphthoquinones retain efficacy against mutant targets both in whole yeast and in enzyme preparations. Ultimately, it is hoped that the results of this study will facilitate the rational use of antipneumocystis drugs.

Grant: 1R01AI047010-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: BERMUDEZ, LUIZ E
Title: MYCOBACTERIAL ENVIRONMENT WITHIN MACROPHAGES
Institution: CALIFORNIA PACIFIC MED CTR-PACIFIC SAN FRANCISCO, CA
CAMP
Project Period: 2000/05/15-2004/04/30

Mycobacterium avium and *Mycobacterium tuberculosis* are intracellular pathogens that infect mononuclear phagocytes and survive within tissue macrophages. Tuberculosis is responsible for millions of deaths annually, and *M. avium* is the most common pathogen causing systemic disease in AIDS patients. Both bacteria survive inside membrane-bound vacuoles in macrophages. The intracellular localization of these organisms suggest that they are well adapted for survival in the host and that they probably can obtain sufficient amounts of nutrients inside the host cells. The presence or absence of elements, such as iron, in the environment is known to influence gene expression in bacteria. It has been assumed that macrophage vacuoles are deficient in iron, as a host defense mechanism against infection. Improving our understanding about the environment within the mycobacterial vacuole and how mycobacteria respond to the conditions present in the vacuoles will provide new insights into pathogenic mechanisms. We propose to: (1) Determine the presence and total concentration as well as the available concentration of important elements such as iron, potassium, phosphorus, sulphur, calcium, magnesium, manganese, copper and zinc in *M. avium* vacuole in macrophages and dendritic cells compared to *M. smegmatis* vacuoles by using two methods: (a) hard X-ray microscopy, and (b) bacterial reporter systems. (2a) Establishing the role of the environment within mycobacterial vacuoles on the expression of mycobacterial putative virulence determinants; (b) determine the importance of these determinants in virulence. This is the first time that we will be able to obtain data on the concentrations of single elements in mycobacterial phagosomes. We believe that the proposed studies will generate important information about the phagosome environment and the mycobacterial response to it.

Grant: 1R01AI047012-01
Program Director: LAUGHON, BARBARA E.
Principal Investigator: RUSSELL, DAVID G PHD PARASITOLOGY, OTH
Title: GLYOXYLATE SHUNT AND PARASITISM BY MYCOBACTERIUM SPP
Institution: WASHINGTON UNIVERSITY ST. LOUIS, MO
Project Period: 2000/01/15-2000/06/30

Mycobacterium tuberculosis is the largest single infectious disease killer in the world today. Its penetrance of the human population is due its ability to cause persistent infections in the human host. Work from our laboratory and several other labs have reported enhanced levels of isocitrate lyase expression in intracellular or dormant bacilli. Isocitrate lyase is the first enzyme of the glyoxylate shunt pathway which exploits acetate or long chain fatty acids as an alternate carbon source. ICL competes with isocitrate dehydrogenase to divert the flux of carbon away from the TCA cycle and into this alternative pathway of carbon acquisition. We propose to examine the role of the glyoxylate shunt and its potential as a drug target. These are the specific goals of this project. 1. Identification of the environmental stimuli responsible for upregulation of ICL expression. We will examine the culture conditions required to induce maximal expression of ICL. In addition we will use the icl promoter to drive expression of ICL: GFP and LacZ constructs to probe the intracellular environment to determine what effect changes in immune status have on ICL expression. 2. The role anaplerotic metabolism in sustaining a latent infection. We shall use both the ICL: GFP construct and antibodies against ICL and malate synthase to probe the expression of these enzymes in murine infections. This work will be undertaken in collaboration with Dr McKinney in different KO mice with different mycobacterial mutants. 3. Identification of compounds inhibitory to ICL and malate synthase activities and their development as antimycobacterial agents. We will use the recombinant enzymes to screen drug libraries at GlaxoWellcome. Structural information generated by Dr. Sacchettini will be central to the development of effective inhibitors against these enzymes.

Grant: 1R01AI047136-01
Program Director: KLEIN, DAVID L
Principal Investigator: REASON, DONALD C PHD
Title: MOLECULAR DETERMINANTS OF HUMAN PNEUMOCOCCAL IMMUNITY
Institution: CHILDREN'S HOSPITAL & RES CTR AT OAKLAND, CA
OAKLAND
Project Period: 2000/04/01-2005/03/31

Antibodies directed against the capsular polysaccharides of the pathogen *Streptococcus pneumoniae* protect humans against infection, and are elicited by vaccination with polysaccharide or polysaccharide conjugated to protein carriers. In the proposed study the variable region gene usage and junctional diversity of human antibodies specific for *S. pneumoniae* capsular serotypes 6B, 14, and 23F will be determined by repertoire cloning and sequence analysis. The influence of plain polysaccharide and polysaccharide-protein conjugate vaccine formulations on the expressed repertoire will be investigated, and the degree to which these thymus-independent and thymus-dependent forms of the vaccine induce somatic mutations and affinity maturation will be determined. The structural determinants of anti-polysaccharide antibody affinity will be defined by sequence comparison, site directed mutagenesis, and molecular modeling. Sequence-defined Fab fragments will be expressed in vitro, their affinity and fine specificity determined, and the relationship between antibody affinity and protective efficacy established using an in vitro opsonophagocytosis assay. Our overall hypothesis is that the quality of an oligoclonal antibody response, such as that seen in humans to bacterial capsular polysaccharides, is influenced to a greater degree by the affinities of the individual antibody binding domains than would be a polyclonal response. These differences in antibody affinity arise as a consequence of variable region gene usage, junctional diversity, and somatic mutation. The generation of affinity loss variants by somatic mutation could therefore lead to a diminution of overall antibody quality. These studies will determine if antibodies to structurally distinct polysaccharides utilize the same or distinct variable region genes and the degree to which maturation of the response through somatic mutation determines overall affinity of the response. These studies will also determine if the same clonotypes occur in unrelated individuals, and if a single clonotype predominates the response to a given specificity. Defining the relationship between binding site affinity and antibody functional quality will provide better surrogate markers of protective immunity. Understanding the molecular mechanisms that shape the human antibody repertoire to pneumococcal polysaccharides may also suggest strategies that would facilitate the development of more efficacious vaccines.

Grant: 1R01AI047152-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: LIDDINGTON, ROBERT C PHD
Title: STRUCTURAL STUDIES ON THE ANTHRAX LETHAL TOXIN
Institution: BURNHAM INSTITUTE LA JOLLA, CA
Project Period: 2000/04/01-2005/03/31

Description: (Verbatim from the applicant's abstract) Anthrax is an ancient disease that has become a renewed concern owing to its threatened use as a weapon of biological warfare and terrorism. Lethal Toxin is the two-part protein toxin secreted by *Bacillus anthracis*, the causative agent of Anthrax, which is sufficient for death in experimental animals. The purpose of this work is to determine the atomic-level mechanisms of intoxication by Lethal Toxin, which comprises the Protective Antigen (PA, 83 kDa) and the Lethal Factor (LF, 90 kDa). These studies will contribute directly to the development of novel therapeutic agents, both in combating the disease of anthrax itself, and in the field of "targeted toxins." We recently published the crystal structure of PA in its monomeric and water-soluble heptameric forms (PA63). Based on its structure, we have formulated a specific hypothesis of pH-induced conformational change leading to the creation of a membrane-spanning beta-barrel. We will test this hypothesis by determining the crystal structure of PA63 in its membrane-bound conformation. PA63 is the central component of a protein translocation machine that delivers LF into the host cell cytosol. In vitro, LF in combination with PA specifically kills macrophages, although it is able to enter all cell types tested. Recent work has shown that LF is a metalloprotease, and that one of its targets is MAP kinase kinase, although it is not clear yet how this activity is related to its pathogenicity. In order to shed light on the catalytic basis of LF action and its regulation, we will determine its atomic resolution structure and complexes with peptide fragments of its target proteins. These crystal structures will pave the way for studying the 7:7 translocation complex between the PA63 heptamer and Lethal Factor, which will provide testable hypotheses of the translocation process. Although these complexes are very large, our atomic-resolution determinations of the constituent elements, together with the 7-fold symmetry, will allow us to build reliable models even with limited resolution data. The work will complement the biochemical and biophysical experiments of our collaborators, Drs. Stephen Leppla at NIH Bethesda, Philip Hanna at Duke University, R. John Collier at Harvard Medical School and Alok Mitra at Scripps.

Grant: 1R01AI047163-01
Program Director: LANG, DENNIS R
Principal Investigator: GHOSH, PARTHO BS
Title: CRYSTAL STRUCTURES OF BACTERIAL PATHOGEN PROTEINS
Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA
Project Period: 2000/05/01-2005/04/30

Description: (Verbatim from the applicant's abstract) The broad, long-term objectives of this proposal are to understand the structural steps required for bacterial pathogens to invade mammalian host cells. Host cell invasion is a critical step in the life cycle of intracellular pathogens and parasites, which are major causes of human morbidity and mortality. The mechanism of cell invasion is being investigated in the pathogen *Listeria monocytogenes*, a cause of recent outbreaks of human illness and death. A protein attached to the cell wall of *L. monocytogenes*, internalin B (67 kD), is solely responsible for triggering the uptake of the bacterium into several nonphagocytic mammalian cell types. Included among these are hepatocytes, which are the major locus of bacterial proliferation in vivo. Internalin B acts by binding to a mammalian receptor, gClq receptor (gClq-R), and activating host phosphoinositide (PI) 3-kinase, leading to induction of phagocytosis. A 25 kD mammalian cell effector domain of internalin B is necessary and sufficient to activate PI-3 kinase, whereas the intact molecule is required for bacterial uptake. How internalin B activates host signaling pathways and causes uptake is not understood structurally. The specific aims of the proposal are to: (1) Determine the structure of the 25 kD effector domain of internalin B. Crystals of the 25 kD effector domain that diffract x-rays to 1.5 Å resolution have been grown and heavy-atom phasing has been achieved, allowing an interpretable electron density map to be calculated. (2) Determine the structure of intact internalin B. Crystals of intact internalin B that diffract x-rays to 3.15 Å resolution have been grown and phasing information is being sought. (3) Co-crystallize and determine the structure of internalin B bound to gClq-R. For these studies, a number of internalin B constructs are available in milligram quantities, as is gClq-R in a form that crystallizes. The proposed structures are important to revealing the stereochemical basis by which internalin B induces phagocytosis and causes host cell invasion. This knowledge will be generally applicable to devising strategies to combat *L. monocytogenes* and other intracellular pathogens.

Grant: 1R01AI047171-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: BEHAR, SAMUEL M MD
Title: ROLE OF THE ALPHAE INTEGRIN IN IMMUNITY TO TUBERCULOSIS
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 2000/09/30-2005/07/31

DESCRIPTION (Adapted from the Applicant's Abstract): The integrin alphaEbeta7 is a cell surface protein that is expressed preferentially on T cells in the mucosal epithelium. While it is appreciated that alphaEbeta7 mediates cell adhesion, and plays a role in the homing of T cells to the respiratory and intestinal epithelium, preliminary studies suggest that signaling via alphaEbeta7 may be critical in the differentiation of CD4 + T cells into TH2 cells. This alteration in T cell cytokine production is biologically significant since in a murine model of ovalbumin induced pulmonary hypersensitivity, mice receiving anti-alphaE mAb or mice genetically deficient in alphaE have decreased cellularity of the bronchoalveolar lavage fluid, lower levels of TH2 cytokines, and less airway hyperresponsiveness. The investigators' hypothesis is that T cell signaling through the alphaEbeta7 integrin is critical in the generation of TH2 responses. In the absence of alphaE, the TH1 response is enhanced as a consequence of a shift in the TH1 / TH2 balance. Since TH1 cytokines are critical to the control and resolution of infections caused by intracellular microbial pathogens, they predicted that blockade or deletion of alphaE may augment the resistance to these infections. Consistent with this novel function of the alphaEbeta7 integrin, their preliminary data indicates that the alphaE deficient mouse is more resistant to the development of tuberculosis. The increased resistance of the alphaE deficient mouse to tuberculosis defines alphaE as a susceptibility gene. This proposal will determine how alphaEbeta7 modifies the susceptibility to Mycobacterium tuberculosis (Erdman) in mice that are inoculated by the respiratory route using an aerosol delivery system. The specific aims are: 1) Assess the pathogenesis of tuberculosis in alphaE deficient (alphaE^{-/-}) and wild type (alphaE^{+/+}) BALB/c mice; 2) Examine whether the cytokine response to M. tuberculosis is altered in alphaE deficient mice; and 3) Investigate potential mechanisms by which alphaEbeta7 may affect susceptibility to tuberculosis.

Grant: 1R01AI047254-01
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: JUDD, RALPH C PHD
MICROBIOLOGY: BACTERIOLOGY
Title: VACCINE POTENTIAL OF GONOCOCCAL OMP85
Institution: UNIVERSITY OF MONTANA MISSOULA, MT
Project Period: 2000/04/15-2002/03/31

DESCRIPTION: (Adapted from the Applicant's Abstract) *Neisseria gonorrhoeae* infects up to half a million people yearly in the US, causing a variety of diseases. Treatment of these diseases costs billions of dollars each year. Furthermore, inflammation from gonococcal infection increases the risk of transmitting and acquiring other sexually transmitted disease agents such as HIV. Despite a pressing need and decades of active research, an effective gonococcal vaccine has yet to be developed. One of the barriers to a gonococcal vaccine is the extraordinary variation of the most abundant and immunodominant gonococcal surface components. There may be less abundant surface molecules that are invariant. Recombinant DNA methods make it practical to develop subunit vaccines that focus immune responses to these less abundant, invariant molecules. The goal of this application is to evaluate the subunit vaccine potential of a particularly promising 85kDA outer membrane protein (OMP85) of *Neisseria gonorrhoeae*. Preliminary data indicated that Omp85 was invariant and universally expressed in tested strains. Antibodies to Omp85 homologues protected against infection with *Haemophilus influenzae* and *Pasteurella multocida*. The hypotheses to be tested are that Omp85 is invariantly and universally expressed in *N. gonorrhoeae* and that antisera directed against Omp85 are bactericidal and interfere with gonococcal adherence to, and/or penetration of, human epithelial cells. The following specific aims will test the hypotheses: (1) the variability of Omp85 among gonococcal strains will be determined. (2) Immunological characteristics of Omp85 will be evaluated. (3) Surface reactive antibodies to Omp85 will be used in bactericidal and cell adherence/invasion assays to determine if anti-Omp85 antibodies are bactericidal and if they interfere with gonococcal-host cell interactions. Completion of the specific aims will demonstrate the vaccine potential of Omp85. If the hypotheses are supported then Omp85 may be a valuable vaccine molecule similar to the related protective antigens in *H. influenzae* and *P. multocida*.

Grant: 1R01AI047255-01
Program Director: KLEIN, DAVID L
Principal Investigator: HARDING, CLIFFORD V MD OTHER AREAS
Title: CPG DNA ADJUVANTS AND VACCINES FOR ENCAPSULATED BACTERIA
Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH
Project Period: 2000/09/29-2005/07/31

DESCRIPTION: (Adapted from the Applicant's Abstract): CpG oligodeoxynucleotides (ODN) have immunomodulatory effects that may be useful for many future vaccine applications. The goal of this proposal is to understand how CpG ODN alter antigen processing and presentation of peptides to T cells. The project will also investigate how CpG ODN alter humoral immunity to polysaccharide Ags, as induced by immunization with either unconjugated PS or PS-protein conjugate vaccines. Aim 1: To determine the effect of CpG ODN on the ability of Ag presenting cells to process Ag and stimulate T cell responses to protein Ags. It is hypothesized that CpG ODN enhance Ag processing by dendritic cells and B cells. Investigators will determine the effects of CpG ODN on the ability of these cells to process and present exogenous protein Ags, including CRM 197, the carrier protein for glycoconjugate vaccines studied in Aims 2 and 3. Mechanisms for these effects will be explored, including the influence of CpG ODN on factors such as MHC-II synthesis and expression, half-life of peptide:MHC-II complexes, and expression of Ag processing components. Aim 2: To explore the adjuvant effects of CpG ODN on responses to PS and peptide epitopes of glycoconjugate vaccines, primarily using an experimental vaccine for *Streptococcus pneumoniae*. It is suggested that CpG ODN will enhance Ab responses to PS epitopes of glycoconjugate vaccines and alter the Ab isotypes that are elicited (e.g., to induce IgG2a and IgG3 responses in mice). The mechanisms of these effects will be determined including the roles of cytokines and T cells. Aim 3: Experiments will test whether CpG ODN can act as effective adjuvants in concert with vaccines containing only unconjugated PS immunogen to enhance PS-specific IgM and IgG1 responses and induce PS-specific Ab of other isotypes. Mechanisms of these effects will be determined (e.g., roles of T cells and cytokines). Understanding the modulation of Ag presenting cells by CpG ODN would increase our understanding of the basic mechanisms of adjuvant function for CpG ODN. The ability of CpG ODN to enhance humoral immunity to PS Ags would allow the development of improved vaccines for encapsulated bacteria.

Grant: 1R01AI047260-01
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: SO, MAGDALENE Y PHD
MICROBIOLOGY: BACTERIOLOGY
Title: GENETIC DETERMINANTS FOR GONOCOCCAL TRANSCYTOSIS
Institution: OREGON HEALTH & SCIENCE UNIVERSITY PORTLAND, OR
Project Period: 2000/06/01-2005/05/31

N. gonorrhoeae (GC) infects the mucosal epithelium of the human urogenital tract. It adheres to and invades epithelial cells in a multistep manner. Several bacterial ligands and their cognate epithelial cell receptors have been identified, and the initial events in adhesion and invasion are beginning to be understood. GC next traverses the epithelial cell and exits into the subepithelial matrix. This is a slow process requiring 36 - 48 hours, and the molecular mechanisms underlying it are unknown. We are interested in GC transcellular trafficking, or transcytosis, and have taken a genetic approach to study the process. We first adapted the polarized T84 epithelial cell system as a model epithelial barrier to study GC transcytosis. We then used this system to screen a random bank of mTn-generated GC mutants for fast-trafficking mutants. Four mutants with mTn insertions in three genetic loci were identified in this initial screen. Backcrosses of these mutations show that the fast- trafficking phenotype segregated with the mTn insertion. These mutants do not adhere to or invade cells more quickly, nor do they affect the integrity of the epithelial barrier. These mutants are therefore aberrant in the transcellular trafficking process, not in the initial steps of colonization. Preliminary studies indicate that the loci are likely to play a regulatory role in transcytosis. One mutant is deregulated in its growth within two types of human epithelial cells; its extracellular growth in liquid medium is normal. In this grant application, we propose to further characterize these three loci in order to elucidate their role in GC transcellular trafficking. We also propose to screen the rest of the GC mutant bank for additional fast-trafficking mutants and to characterize their mutated genes. Such studies should shed light on the genetic regulation of the transcytosis process, and hopefully guide the design of novel pharmacologic agents against intracellular GC.

Grant: 1R01AI047276-01
Program Director: MILLER, MARISSA A.
Principal Investigator: BAYLEY, HAGAN P
Title: MEMBRANE PROTEIN ENGINEERING BY TARGETED MODIFICATION
Institution: TEXAS A&M UNIVERSITY HEALTH SCIENCE COLLEGE STATION, TX
CTR
Project Period: 2000/03/15-2001/02/28

Dramatic modifications and extensions of the functional properties of a transmembrane pore will be brought about by targeted modification of staphylococcal alpha-hemolysin (alphaHL). AlphaHL is secreted by *Staphylococcus aureus* is a water-soluble, 293-residue polypeptide, which forms heptameric pores in lipid bilayers. It is an excellent target for the proposed studies: three dimensional structures are available, the protein is robust and can be obtained in abundance, it self-assembles into membranes with a fixed subunit stoichiometry and in a single orientation, a large collection of mutants is available, the protein tolerates extreme manipulations in structure, heteroheptamers can be prepared and purified. Further, the functional properties of the pore can be examined in intricate detail by single channel recording. Given its high conductance, prolonged open stage and weak ion selectivity, alphaHL constitutes a blank slate for protein engineering. AlphaHL will be radically remodeled by targeted non-covalent and covalent modifications with cyclodextrins, cyclic peptides, responsive polymers, oligonucleotides and a bipartite chelator. The manipulations proposed, including the alteration of the inside of a protein cavity, have not been carried out previously on membrane proteins and have been performed only rarely on any class of protein. Importantly, we will not be content to demonstrate folding and assembly alone; our focus will be on function, especially alterations in unitary conductance, ion selectivity, and susceptibility to channel blockers. Expected outcomes are: (i) a better understanding of the fundamental properties of transmembrane channels; (ii) the creation of pores with new properties not found in nature; (iii) a better understanding of the rules governing the construction of modular nanostructures from biological macromolecules that do not normally interact. The work will impact healthcare, by providing new technology for biosensors, cell preservation, biotherapeutics and drug delivery.

Grant: 1R01AI047311-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: PAVELKA, MARTIN S BS
Title: BIOSYNTHESIS OF THE MYCOBACTERIAL PEPTIDOGLYCAN
Institution: UNIVERSITY OF ROCHESTER ROCHESTER, NY
Project Period: 2000/09/30-2005/07/31

DESCRIPTION (Adapted from the Applicant's Abstract): Tuberculosis is the leading cause of death in adults due to an infectious organism. By the end of this century there may be as many as 90 million new cases of tuberculosis resulting in up to 30 million deaths. The failure of antimicrobial therapy and the dangerous association between tuberculosis and AIDS have brought renewed interest in studying *M. tuberculosis*, the organism responsible for this disease. A better understanding of the basic biology of the organism and the development of new anti-mycobacterial drugs are important goals of mycobacterial research. The cell envelope is an outstanding characteristic of the mycobacteria, consisting of a variety of polysaccharides such as arabinogalactan, lipoarabinomannan, and peptidoglycan, along with several different types of lipids including various glycolipids and the mycolic acids. The peptidoglycan layer of the cell envelope serves as the anchor for the principal components of the cell envelope and provides shape and structural integrity to the cell. The long-term goal of this proposal is a deeper understanding of the biosynthesis and assembly of the mycobacterial cell envelope. The specific goal of this proposal is to understand more about the genetics and biosynthesis of the peptidoglycan layer of the envelope. The specific aims of this proposal are: 1) Determining the significance of N-glycolylation of the mycobacterial peptidoglycan. 2) Investigating the architecture of the peptidoglycan and the role it plays in the organization of the cell envelope. 3) Using beta-lactam antibiotics as tools to probe mycobacterial peptidoglycan biosynthesis. For the aims of this proposal, the PI will study *M. tuberculosis* and *M. smegmatis* as a model organism using the techniques of classical bacterial genetics, molecular biology and biochemistry.

Grant: 1R01AI047383-01
Program Director: LANG, DENNIS R
Principal Investigator: WADE, WILLIAM F PHD
Title: VIBRIO CHOLERAE TCP AND LPS SUBUNIT VACCINE, EPITOPES AN
Institution: DARTMOUTH COLLEGE HANOVER, NH
Project Period: 2000/04/01-2005/03/31

Cholera is an acute diarrheal disease caused by the gram-negative bacterium, *Vibrio cholerae*. Following ingestion of contaminated food or water, bacteria colonize the small intestine and secrete cholera toxin, which is responsible for the extensive loss of fluid and electrolytes from infected individuals. Cholera remains a worldwide problem. Although a number of live, attenuated or killed whole cell vaccine formulations have been tested, none have proven successful enough to result in their widespread use. This grant proposal focuses on new approaches to develop a cholera subunit vaccine formulation based on the current understanding of *V. cholerae* colonization, pathogenesis, and human immune responses to infection. The strategy will incorporate features of the highly successful *Haemophilus influenzae* type b and pertussis vaccines that utilize defined surface virulence determinants, colonization factors, and toxoids to achieve long-lasting protection. The present proposal will focus on formulations that include the toxin coregulated pilus (TCP) colonization factor, detoxified LPS, and the adjuvant cholerae toxin. The general goal for these studies is to define the most effective combination of TCP and LPS antigens that provide for protective humoral responses. We will characterize in detail the B cell epitopes for these antigens and determine which epitopes are minimally required for effective immunity. Studies will extend this information to human sera and demonstrate that the epitopes we have identified are operational in the field. Using a well-established mouse model, we will quickly be able to define the immunization regimen and immunogens that are likely candidates for extension into human trials. The proposed research formally brings together the expertise from two research groups at Dartmouth Medical School. Dr. William Wade is a molecular immunologist with training in structural and functional analyses of proteins as they relate to antigen presentation. His research focuses on methodologies to optimize the immunogenicity and delivery of antigens. Dr. Ronald Taylor's research focuses on the molecular basis of *V. cholerae* pathogenesis. He discovered TCP and his group has been instrumental in characterizing the *tcpA* gene and corresponding pilin protein as well as demonstrating that TCP is the major *V. cholerae* colonization factor and a protective antigen. Together, the expertise of these two research groups will provide a unique opportunity to develop and evaluate *V. cholerae* subunit vaccines using new approaches.

Grant: 1R01AI047407-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: RIKIHISA, YASUKO PHD
MICROBIOLOGY: BACTERIOLOGY
Title: GENE EXPRESSION BY HUMAN GRANULOCYTIC EHRLICHIOSIS AG
Institution: OHIO STATE UNIVERSITY COLUMBUS, OH
Project Period: 2000/05/01-2005/04/30

DESCRIPTION (Adapted from Applicant's Abstract): Human granulocytic ehrlichiosis (HGE) is a new tick-borne zoonosis that is increasingly recognized as a threat to public health in the United States. HGE is a systemic febrile illness often accompanied by hematological abnormalities including leukopenia and thrombocytopenia. It frequently requires prolonged hospitalization and when the treatment is delayed due to misdiagnosis or in immunocompromised patients, HGE can be fatal. HGE is caused by infection of peripheral blood granulocytes with an obligate intracellular bacterium, an ehrlichia sp. called an HGE agent. The investigators previously showed that a family of 44-kDa-range major outer membrane proteins of the HGE agent is the immunodominant antigen in human infection. They demonstrated that 44-kDa proteins (P44s) are encoded by a multigene family. Their recent study indicates that there are a total of approximately 18 copies of p44 genes and the HGE agent in a human promyelocytic leukemia cell line, HL-60, expresses 5 of them. These p44 genes are expressed at different sites that are widely distributed throughout the genome, suggesting a potential unique mechanism of providing antigenic diversity in the HGE agent. This system of P44 major surface proteins may provide an instructive model for studying adaptive genetic strategies, and fundamental surface properties, of several ehrlichial species contributing to human and animal diseases. Structural and combinatorial variation in P44 profiles expressed on the unique intracellular bacterial surface, may profoundly affect in-host adaptive capabilities of ehrlichiae. Their specific aims will address important questions in the understanding of this prototype system, including: (1) the degree, pattern and variation of p44 genes expressed by the HGE agent in its different hosts (tick vector, rodent reservoir, and accidental host), (2) the range of variation in the natural repertoire of p44 genes and expression among HGE agent strains, (3) the antibody response to each P44 protein in infected animals and humans, and (4) P44 protein interactions and p44 gene expression mechanisms. Approaches include RT-PCR and sequencing-based characterization of p44 genes expressed by HGE agents in experimentally infected animals and ticks and in several human isolates, cloning the p44 genes expressed, analysis of antibody responses to individual P44-specific peptides, analysis of P44 complexes and a unique mRNA splicing mechanism. These efforts are expected to reveal an important role for the p44-multigene family in strategies of adaptive surface variation and avoidance of host immune responses.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01AI047608-01
Program Director: SAVARESE, BARBARA M.
Principal Investigator: WAWER, MARIA J MD INTERNAL
MED:INFECTIOUS DISEASE
Title: BV ETIOLOGY, NATURAL HISTORY, AND SEXUAL TRANSMISSION
Institution: COLUMBIA UNIVERSITY HEALTH SCIENCES NEW YORK, NY
Project Period: 2000/09/01-2005/03/31

The proposed study will test hypotheses regarding microbiological, virological and behavioral risk factors for the development of bacterial vaginosis (BV), a common vaginal condition which is increasingly recognized as having serious health sequelae, including adverse pregnancy outcomes, pelvic inflammatory disease, and increased risk of HIV infection. The etiology and natural history of BV are poorly understood. The study will be conducted in Rakai District, Uganda, where approximately 50 percent in the general population of women of reproductive age have BV. We propose to conduct two complementary research activities: I, a BV natural history study in a cohort of 250 women (with and without BV, HIV and prior sexual experience), and II, a study in 50 polygamous family units which will enrol the husband, his wives and other women residing in the household. Repeated interview and sample collection in the two studies will be used to assess transition probabilities of BV onset, persistence, regression and recurrence in relation to: a) detailed sociodemographic, behavioral and health data; b) vaginal microflora, particularly Lactobacillus species (which will be characterized using DNA homology and assessed for H₂O₂ production) and c) the potential presence of lactobacillus bacteriophages, whose possible role in Lactobacillus depletion will be explored. In the polygamous household study, we will determine whether factors associated with normal vaginal flora or with BV (including lactobacilli, anaerobes and phages) may be transmitted sexually or via close household contact such as through the sharing of bathing utensils or water, by comparing women with a polygamous sexual network to other women within the household. The Rakai population offers a unique opportunity to assess BV. We previously enrolled and followed approximately 7,000 women in a population-based trial of STD control for AIDS prevention, have documented increased risk of HIV and adverse birth outcomes in women with BV, and have evidence of improved pregnancy outcomes with STD/BV treatment. The proposed study will provide unique epidemiological, microbiological and virological data regarding normal vaginal flora in this rural African population, the natural history of BV, and on potential causes of this prevalent condition. Such information will be critical for the design of future BV prevention, treatment and control trials, including selection of interventions to be tested, sample size requirements and definition of study end points.

Grant: 1R01AI047694-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: SILVERSTEIN, SAMUEL C MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC
Title: ARYL BRANCHED CHAIN ACYL COA ESTERS INHIBIT MYCOBACTERIU
Institution: COLUMBIA UNIVERSITY HEALTH SCIENCES NEW YORK, NY
Project Period: 2000/04/01-2004/03/31

All current antibiotics, with the exception of isoniazid (INH) and ethionamide, inhibit bacterial growth by inhibiting bacterial RNA, DNA, protein or cell wall synthesis. While bacteria and mammalian cells synthesize lipids via pathways that are similar in principle, the enzymes that catalyze bacterial lipid synthesis differ in fundamental respects from their mammalian counterparts. Bacteria, especially *M. tuberculosis* (*M.tb.*), contain unique lipids not found in mammalian cells. We have discovered that the lypolipidemic drug gemfibrozil (GFZ), which has been used safely in humans for >20 years, blocks growth of 27 different pan-drug sensitive and multi-drug resistant strains of *M.tb.* as well as 10 other species of bacteria. GFZ exerts a bactericidal effect on *L. pneumophila*, both in bacterial growth medium and in macrophages. Thus metabolites found in mammalian cells do not block GFZ's inhibitory effect on *L. pneumophila*. We have screened >10(12) *L. pneumophila* colonies but have found no GFZ-resistant mutants. This suggests that GFZ acts on highly conserved, hard to mutate enzyme(s). The 3- and 6-propylene analog of GFZ are 5- fold more potent than GFZ in blocking 14C-acetate incorporation into *L. pneumophila* lipids. Other fibric acids such as clofibrate and bezafibrate, are ineffective. We have identified an *L. pneumophila* enoyl reductase (*Lpn FabX*) that is GFZ's presumptive target, purified the enzyme and showed it is competitively inhibited by GFZ's CoA adduct (GFZ-CoA), but not by GFZ. GFZ-CoA also competitively inhibits *InhA*, the *M.tb.* enoyl reductase that is a target of INH. *L. pneumophila* converts 3H-GFZ to 3H- GFZ-CoA in vivo. GFZ-CoA is the first competitive inhibitor of a bacterial enoyl reductase to be identified. Funds are requested to explore the mechanisms by which GFZ inhibits *M.tb* growth, and to test the effects of GFZ and of its 3- and 6-propylene analogs, alone and in combination with other anti-tuberculosis drugs, on *M.tb* growth in macrophages and in mice.

Grant: 1R01AI047818-01
Program Director: MILLER, MARISSA A.
Principal Investigator: MOORE, BRADLEY S BS
Title: ENGINEERING DIVERSITY WITH ENTEROCIN BIOSYNTHESIS GENE
Institution: UNIVERSITY OF ARIZONA TUCSON, AZ
Project Period: 2000/08/01-2005/07/31

A very important task in the ongoing search for new clinically useful drugs is the generation of large numbers of structurally diverse compounds. These molecules are required for screening by high-throughput bioassays in the discovery of new lead drug candidates. Combinatorial biosynthesis, in which nature's chemical capabilities are exploited in a combinatorial "mix-and-match" fashion, has generated libraries of novel molecules representing great structural diversity which are not available naturally or generated through combinatorial synthesis. The marine bacterium "*Streptomyces maritimus*" is uniquely capable of naturally producing a diverse series of structurally uncommon bacteriostatic polyketides known as the enterocins and wailupemycins. Cloning, sequencing, and heterologous expression of the single biosynthesis gene cluster (*enc*) for these molecules revealed an unprecedented iterative type II polyketide synthase (PKS) system. Polyketide structural variability is probably achieved by the lack of a dedicated cyclase and the action of a rare enzyme-mediated Favorskii rearrangement. The novel architecture of this natural PKS gene set furnishes insight into engineering molecular diversity through genetic recombination and provides the major rationale for the proposed project. In this grant application, we propose to continue our biosynthetic analysis of the enterocin family at the chemical, biochemical and genetic levels. Our primary goal is to fully characterize this natural biosynthetically diverse pathway in order to engineer mutant organisms harboring and expressing altered gene clusters in which specific biosynthetic genes have been deleted, added or replaced with homologous genes from other biosynthetic pathways. The resulting recombinant will be chemically analyzed for the production of polyketide analogs and assayed for biological activities. Biosynthetic enzymes that will be exploited in recombinant systems include: *EncM*, a flavin-dependent oxygenase that is putatively involved in the derailment of the minimal *enc* PKS from generating aromatic endproducts, the enzymes involved in the synthesis and incorporation of the rare benzoyl-CoA PKS starter unit, the regiospecific P450 monooxygenase *EncR*, and the substrate tolerant methyltransferase *EncK*. Secondly, the "*S. Maritimus*" *enc* genes and their sequences will be used to analyze an enterocin-containing marine invertebrate and its associated microflora for the presence of homologous genes in order to model marine microbial-invertebrate symbiosis.

Grant: 1R01AI047853-01
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: ZHANG, YOU-XUN PHD
Title: DISULFIDE BRIDGE EPITOPES IN CHLAMYDIAL MOMP
Institution: BOSTON MEDICAL CENTER BOSTON, MA
Project Period: 2000/06/01-2005/05/31

DESCRIPTION (Adapted from the Applicant's Abstract): The chlamydial major outer membrane protein is the most abundant surface protein on *C. trachomatis* and its well-defined sequence variability accounts for the separation of *C. trachomatis* isolates into 18 distinct serovars. *C. trachomatis* MOMP also confers structural rigidity to the infectious form of the organism and exhibits characteristics consistent with porin activity. Evidence also exists to suggest that MOMP may contribute to chlamydial invasion into susceptible eukaryotic host cells, yet little is known regarding the conformational features of this molecule. The goals of the work proposed in this application are to determine the location of intra- and intermolecular MOMP disulfide bridges and establish the conformational features of surface exposed MOMP epitopes using site-specific mutagenesis and molecular modeling techniques.

Grant: 1R01AI047885-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: RIKIHISA, YASUKO PHD
MICROBIOLOGY: BACTERIOLOGY
Title: COMPARISON OF 3 HUMAN EHRLICHIOSIS GENOME SEQUENCES
Institution: OHIO STATE UNIVERSITY COLUMBUS, OH
Project Period: 2000/08/01-2003/07/31

DESCRIPTION (Adapted from the Applicant's Abstract): Ehrlichiae are obligate intracellular gram-negative bacteria that replicate in leukocytes. Several Ehrlichia spp. are now known to cause emerging vector-borne zoonoses in the United States, including a new granulocytotropic ehrlichia, the human granulocytic ehrlichiosis (HGE) agent, and Ehrlichia chaffeensis, a new monocytotropic ehrlichia causes human monocytic ehrlichiosis. E. sennetsu is another monocytotropic agent and the first human ehrlichial pathogen discovered. These organisms often cause severe systemic febrile illness accompanied by hematological abnormalities. The mechanisms responsible for pathogenesis, and the development of protective immunity are poorly understood. The research progress on this group of bacteria has been hampered because cultivation and purification of ehrlichiae are difficult. As these organisms are obligate intracellular pathogens, conventional microbiological and molecular genetic approaches have not been feasible. The overall goal of the proposed study is to sequence and annotate the genome of three human pathogens: the HGE agent, E. chaffeensis and E. sennetsu, each representing three genetically divergent groups of all Ehrlichia spp. (up to 15 percent divergent in 16S rRNA gene sequences). The genomes of the HGE agent, E. chaffeensis and E. sennetsu are 1.5, 1.2 and 0.9 Mb in size, respectively. Thus, genome sequencing of these organisms will yield immediate answers to a number of important questions. For example, it will be possible to identify which metabolic activities are present, whether lipid A or LPS is present, and what types of iron uptake system exists in Ehrlichia spp. The rationale for choosing three representative Ehrlichia species is that they are sufficiently different both with respect to genome issues as well as the interaction with the host cells to warrant a complete comparative analysis of all three genomes. A whole genome shotgun approach will be employed. Additionally, a large fragment library will be prepared in a BAC vector to serve as scaffolding for the ordering of contigs and for gap closure. Annotation of the assembled sequence will be performed after assembly. Genomes will be compared to determine the metabolic capabilities of each organism as well as to characterize unique and conserved virulence determinants. Sequencing of these three Ehrlichia spp. will provide comprehensive and comparative knowledge on ehrlichial organisms. The data to be obtained along with data accumulating from projects on other obligate intracellular bacteria and vector-borne pathogens will facilitate the development of new testable hypotheses regarding the virulence mechanisms and the development of a rational vaccine candidate, chemotherapy, and differential diagnostic methods.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01AI047997-01
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: ZHONG, GUANGMING PHD
Title: MECHANISMS OF CHLAMYDIAL EVASION OF IMMUNE RECOGNATION
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR SAN SAN ANTONIO, TX
ANT
Project Period: 2000/07/15-2005/06/30

Human chlamydial infections are recognized as the leading cause of many important sexually transmitted diseases worldwide. The development of chlamydial diseases is largely due to persistent intracellular infection by the organisms. Chlamydial evasion of host immune response may contribute to the persistence of chlamydial infection. We found that chlamydia has evolved the ability to evade immune recognition by inhibiting both MHC class I and class II antigen expression, which is correlated with degradation of transcription factors required for MHC gene activation. We further found that a chlamydia-specific proteasome-like activity in chlamydia-infected cell cytosolic fraction is responsible for the transcription factor degradation. We hypothesize that chlamydia may secrete a factor(s) with the unique proteasome-like activity into host cell cytosol for evading immune recognition mechanisms. We designated this factor as chlamydial proteasome-like activity factor (CPAF). To test our hypothesis, we propose (1) to purify and to identify CPAF, (2) to understand how CPAF works including determining its subcellular location, intracellular trafficking, binding specificity, potential cofactors and function domains and (3) to search for inhibitors for blocking the enzymatic activity of CPAF using a phage-displayed peptide library plus affinity selection approach. These studies will provide essential information for understanding the molecular mechanisms of chlamydial pathogenesis and developing effective strategies for preventing chlamydial persistence and controlling chlamydia-induced diseases.

Grant: 1R01AI048066-01
Program Director: KLEIN, DAVID L
Principal Investigator: BARENKAMP, STEPHEN J MD
Title: H. INFLUENZAE HMW ADHESION PROTEINS IN HOST IMMUNITY
Institution: ST. LOUIS UNIVERSITY ST. LOUIS, MO
Project Period: 2000/07/01-2003/06/30

Otitis media and other illnesses caused by nontypable *Haemophilus influenzae* (NTHI) remain significant health problems for children in this country and elsewhere in the world. The long-term objectives of this project are to identify those surface-exposed bacterial antigens of NTHI that are important in a protective host immune response and ultimately to address the question of whether or not a vaccine composed of such antigens would be effective in the prevention of disease. In previous work, we identified the HMW1/HMW2 family of proteins as major targets of the human serum antibody response following natural infection and colonization. Furthermore, we demonstrated a critical role for these proteins in adhesion of NTHI to eukaryotic cells. With respect to the potential of these proteins as vaccine candidates, we demonstrated that immunization of chinchillas with an HMW1/HMW2 mixture provided protection against experimental NTHI otitis media caused by the homologous strain. Finally, in our preliminary studies, we demonstrated that adult human serum antibodies specific for the HMW1/HMW2 family of proteins mediate opsonophagocytic activity against both homologous and heterologous strains. We propose to build on these earlier studies with the following specific aims. We will characterize the contribution of antibodies produced against the HMW1/HMW2-like proteins to the opsonophagocytic activity that develops in convalescent sera of children with acute NTHI otitis media. We will map those regions of the HMW1/HMW2-like proteins that express epitopes recognized by antibody capable of mediating opsonophagocytic activity against both homologous and heterologous strains. Finally, we will assess the ability of recombinant proteins that express epitopes recognized by antibodies mediating opsonophagocytic activity against homologous and heterologous strains to provide protection against disease in the chinchilla model of experimental NTHI otitis media. The knowledge gained from these studies will provide a clearer picture of the role of antibody to the HMW1/HMW2 proteins in host immunity and may move us closer to the goal of developing vaccines for prevention of nontypable *Haemophilus influenzae* otitis media and other diseases in young children.

Grant: 1R01AI048417-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: RAJAGOPALAN, MALINI PHD
Title: CELL DIVISION IN MYCOBACTERIUM TUBERCULOSIS/FTSZ PROTEIN
Institution: UNIVERSITY OF TEXAS HLTH CTR AT TYLER, TX
TYLER
Project Period: 2000/08/01-2005/07/31

Abstract Text Not Available

Grant: 1R01AI048489-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: YOUNG, JOHN A.T. PHD
Title: STRATEGIES TO INHIBIT CELLULAR UPTAKE OF ANTHRAX TOXIN
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 2000/08/15-2004/07/31

Bacillus anthracis, the causative agent of anthrax, is a spore-forming organism. The ability to prepare anthrax spores inexpensively and deliver them in an aerosol form, and the high mortality rate of inhalation anthrax, have made *B. anthracis* one of the most dreaded agents of biowarfare and bioterrorism. This bacterium encodes two toxins, lethal toxin (LF) and edema toxin (EF), which are collectively called "anthrax toxin" (AT). Lethal toxin is assembled from protective antigen (PA, 83 kDa) and lethal factor (LF, 90 kDa), and as its name implies, is primarily responsible for lethality from anthrax. Edema toxin is assembled from PA and Edema Factor (EF, 89 kDa), and has as its gross manifestation edema at the site of injection. Uptake of each of these toxins is critically dependent upon interaction of PA with its cellular receptor, which leads to receptor-mediated endocytosis followed by toxin translocation into the cytosol across the membrane of an acidic pH endosome. However, the precise understanding of how these toxins are taken up into cells, and the ability to intervene therapeutically in this process, has been severely impaired by not knowing the identity of the PA receptor. Therefore, the primary goal of the proposed research is to isolate and characterize the cellular receptor for PA. To this end, the applicant will use a genetic complementation approach that employs, as recipient cells, mutant CHO-K1 cell lines generated by the applicant and then subjected to a selection protocol to identify those lacking PA receptors. Once this receptor has been identified, the applicant will localize the PA binding domain, characterize the nature of its binding interaction with PA, and attempt to define the minimal cellular components required for AT assembly and translocation across membranes. The applicant will also determine how this receptor is taken up into cells after PA binding and oligomerization, by defining determinants of the receptor and cellular factors that are required for this process. Libraries of chemical compounds will be screened for effective inhibitors of the early steps of AT uptake and translocation. Collectively, these studies will significantly increase the knowledge of how anthrax toxin is taken up into cells and should identify effective chemical inhibitors of this process for future drug development.

Grant: 1R01AI048490-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: SPLITTER, GARY A DVM
Title: BRUCELLA VACCINE FOR BIOTERRORISM
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 2000/07/15-2004/06/30

DESCRIPTION: *Brucella* spp. is a Gram negative facultative intracellular bacterium that induces chronic infectious disease by direct contact or by consumption of animal products. *Brucella* is considered a potential pathogen for bioterrorism. Our long-term goal is to develop a *Brucella* vaccine. Little is known regarding *Brucella* genes encoding proteins that contribute to intracellular survival and virulence. Recently, a *Brucella* promoter trap system has been engineered using the promoterless green fluorescence protein (GFP) gene to identify *Brucella* promoters and associated genes that are activated following intracellular infection. A library of *Brucella* genes activated within 4 hours of macrophage infection has been identified and additional genes are being determined. Also, our previous evidence indicates that CD4+, CD8+ T cells and IFN-gamma are prominent during clearance of acute infection; but little data exist to indicate the immunologic features critical to disease resolution. However, a *Brucella* memory response is a likely foundation for successful vaccination, and immunologic memory is hypothesized to play a key role in protection. Now, strategies will be created to protect interferon regulatory factor-1 gene knockout (IRF-1-r) mice that die within 7-10 days from virulent *Brucella* using attenuated *Brucella* mutants as vaccine candidates, and evaluate the cytokines and cell phenotypes of immune cells to understand the mechanism of protection. In addition, IRF-1-r mice can mount a protective immune response if vaccinated with certain attenuated mutants. The following specific aims are proposed. Specific Aim 1 will identify *Brucella abortus* genes that affect intracellular survival and then engineer those gene deletion mutants of *Brucella*. Novel promoter-gene combinations activated during in vitro macrophage infection will be identified. In preliminary studies, we have isolated a library of important genes, and results obtained from these studies will be used in the design of attenuated mutant *Brucella*. Gene deletion mutants of *Brucella* will be engineered. Attenuated mutant *Brucella* will be used as vaccine candidates in highly susceptible IRF-1-r mice. Specific Aim 2 will evaluate the efficacy of attenuated mutant *Brucella* as potential vaccines and determine the mechanisms responsible for a protective memory response in mice. Protective immunity of selected attenuated mutant *Brucella* in IRF-1-/- and C57BL/6 mice will be evaluated. *Brucella* mutants will be tested for their ability to confer protection to IRF-1-r mice as a rapid screen. Candidate mutants will then be tested in C57BL/6 mice followed by virulent *B. abortus* challenge. The immunologic components that induce protection will be identified using IRF-1-r and C57BL/6 mice. The change in cell phenotypes and cytokines will be monitored throughout the course of vaccination and challenge. Our goal is to use these findings to help develop a vaccine for *Brucella*. Importantly, no *Brucella* vaccine is available for humans, and there is an immediate need to protect the public against *Brucella* that might be used by bioterrorists.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01AI048491-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: STRALEY, SUSAN C PHD BOTANY
NEC:BIOPHYSICS
Title: Early Events in Pneumonic Plague
Institution: UNIVERSITY OF KENTUCKY LEXINGTON, KY
Project Period: 2000/09/25-2003/08/31

Yersinia pestis, the causative agent of pneumonic and bubonic plague, has a high potential for use as a biological warfare or bioterrorism agent. The bacterium is easily cultured and genetically manipulated and can be delivered in aerosolized droplets. The resulting pneumonic plague has a short incubation time and is rapidly and highly fatal. Great potential exists for spread of pneumonic plague from primary infected individuals to their contacts. Currently, there is no available vaccine against pneumonic plague. Research from Dr. Straley's group has revealed two kinds of pathogenic mechanisms that potentially could function early in pneumonic plague. Contrary to reports in the literature, they have found that *Y. pestis* cells invade host epitheloid cells at significant rates. They have also found that *Y. pestis* possesses three quorum-sensing, or cell-density signaling systems. The roles of these two traits in the pathogenesis of pneumonic plague have not been investigated. In fact, relatively few studies of pneumonic plague have been performed. In the interest of identifying early targets for intervention in pneumonic plague, the applicant proposes to characterize host cell invasion and the three quorum-sensing systems in *Y. pestis*. The applicant will use a pneumonic plague mouse model to determine the roles of these traits in virulence. These studies should enhance understanding of the pathogenesis of pneumonic plague and facilitate the development of measures to protect people against possible bioterrorism based on *Y. pestis*. The proposed study is designed to have two lines of research running in parallel so as to gain maximal synergism from the collaboration of three research groups. The specific aims of this application are 1) to characterize the role of invasion in pneumonic plague and 2) to characterize the role of quorum sensing in pneumonic plague.

Grant: 1R01AI048499-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: ROOP, ROY M
Title: BRUCELLA STATIONARY PHASE GENE EXPRESSION AND VIRULENCE
Institution: LOUISIANA STATE UNIV HSC SHREVEPORT SHREVEPORT, LA
Project Period: 2000/07/15-2000/12/31

DESCRIPTION: *Brucella* spp. have several pathogenic properties that make them a serious potential threat for use as agents of biological warfare and bioterrorism. Specifically, they are highly infectious by the aerosol route, they produce a chronic, debilitating disease in humans that is difficult to treat, and there is no safe and effective vaccine available to prevent human brucellosis. Prolonged survival and replication in host macrophages is critical to the capacity of the brucellae to establish and maintain chronic infection in the host. During their long term residence in host macrophages, the brucellae encounter a variety of harsh environmental conditions including nutrient limitation and exposure to reactive oxygen intermediates and acidic pH. Experimental evidence indicates that the *B. abortus* hfq gene product (also known as host factor I, or HF-I) is essential for the capacity of this organism to withstand exposure to these environmental stresses in host macrophages. Based on the well documented function of its enteric counterparts, the Principal Investigator's working hypothesis is that the *B. abortus* hfq gene product performs this function by facilitating optimal translation of the gene encoding a homologue of the stationary phase specific RNA polymerase sigma factor RpoS. The specific aims of this project are: 1) to clone the *B. abortus* rpoS gene, confirm its regulatory link to HF-I, and evaluate its contribution to stationary phase physiology in vitro and virulence in the mouse model; 2) to determine if HF-I and RpoS control stationary phase expression of the *B. abortus* katE and sodC genes, which encode important primary antioxidants linked to virulence in mice; and 3) to identify other HF-I and RpoS-regulated genes in *B. abortus* that play critical roles in the capacity of this bacterium to establish and maintain chronic infection in the murine host. Defining the physiologic state of the intracellular brucellae during chronic infection in the host and elucidating the contributions of individual stationary phase gene products to successful survival and replication in host macrophages should provide important basic information regarding host-pathogen interactions in *Brucella* infections. This information may also be useful for the design of novel vaccine candidates and improved chemotherapeutic approaches.

Grant: 1R01AI048505-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: ARONSON, ARTHUR I PHD
Title: STRATEGIES FOR INACTIVATING BACILLUS ANTHRACIS SPORES
Institution: PURDUE UNIVERSITY WEST LAFAYETTE WEST LAFAYETTE, IN
Project Period: 2000/09/29-2004/08/31

Bacillus anthracis is an animal and human pathogen with potential as a biological warfare agent. It is a spore-forming Bacillus with pathogenicity due to plasmid genes encoding three toxin components and a polyglutamic acid capsule. Its effectiveness as an agent is due to the ease of producing and spreading the spores. These spores are dormant and very resistant to a variety of stress conditions such as heat, ultraviolet radiation, and chemical treatment. The spores can thus remain in the soil or phylloplane for many years. Under suitable conditions, they can germinate and vegetative cells will propagate in the soil and then resporulate. Thus, there is an enormous potential for sustaining and spreading this organism once it has been dispersed. The spore is surrounded by a multilayered proteinaceous coat that accounts for much of its resistance properties and contributes to the capacity of the spore to respond to germinants and propagate as vegetative cells. A detailed understanding of the spore coat structure, how it is assembled, and how it can be specifically disrupted would provide an effective way for controlling this bioweapon. Information gleaned from extensive studies of Bacillus subtilis spore coat assembly and the function of the 25 or so proteins that make up the coat layers of this species will be used to isolate and characterize spore coat protein genes from B. anthracis. This will be done in part by identifying B. subtilis homologues in the B. anthracis genome and in part by reverse genetics using the amino acid sequences of purified B. anthracis coat proteins. These genes will be disrupted and the effects of such null mutations on spore coat assembly and structure determined. Some features of the B. anthracis spore such as germination response and spore coat structure have been shown to vary with the conditions used for spore formation. Most significantly, culture conditions that induce many pathogenicity genes, including the toxin genes, resulted in a major change in the spore coat protein profile. This correlation may reflect coordination between pathogenicity due to toxin synthesis and encapsulation of vegetative cells and special spore properties that could enhance infectivity. The nature of the spore coat changes and their contribution to spore germination and resistance will be examined. This information about spore coat composition and its variation with culture conditions will be exploited to find specific reagents and procedures for destroying the spore or for inhibiting germination. Once such reagents or methods have been identified, they will be tested on spores in various soils that should mimic natural environments and thus the conditions needed for the effective control of this bioweapon.

Grant: 1R01AI048506-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: ZHANG, ZHONG-YIN PHD
Title: YERSINIA PTPASE INHIBITORS AS ANTI-PLAGUE AGENTS
Institution: YESHIVA UNIVERSITY NEW YORK, NY
Project Period: 2000/09/30-2004/07/31

Yersinia pestis was the pathogen responsible for the bubonic plague, also known as the Black Death. Although plague has long been considered a once-vanquished disease, the recent outbreak of the pneumonic plague caused by *Yersinia pestis* in Surat, India, proves that its biological potential can be expressed under appropriate environmental conditions. In addition, there is increasing risk of misuse of infectious agents, such as *Yersinia pestis*, as weapons of terror, as well as instruments of warfare for mass destruction. Thus, there is an urgent need to devise effective protective strategies that could be implemented soon after a bioterrorist attack. Although an understanding of the pathogenic processes induced by *Yersinia pestis* is incomplete, several of the bacterial virulence factors have been located on a naturally occurring 70 kb plasmid. The expression of a set of proteins encoded on the virulence plasmid known as YOPs (for *Yersinia* outer membrane proteins) is correlated with the capacity of the bacterium to avoid host defense mechanisms. One of the YOP proteins, YopH, is a protein tyrosine phosphatase (PTPase). The *yopH* gene is obligatory for pathogenesis, and plasmids that have a nonfunctional *yopH* gene are avirulent, suggesting that the *Yersinia* PTPase activity is essential for bacterial pathogenicity. Thus, specific inhibitors of the *Yersinia* PTPase are expected to be powerful agents to prevent and terminate the dissemination of *Yersinia pestis* infection. A multidisciplinary research program is designed to develop and evaluate specific inhibitors of the PTPase (YopH) from *Yersinia pestis* as novel anti-plague agents. Efficient and specific YopH substrates will be identified using detailed kinetic analysis of the PTPase active site specificity using libraries of aryl phosphates. Potent and selective YopH inhibitors will be prepared by converting the efficient and specific aryl phosphate substrates to their nonhydrolyzable difluorophosphonate derivatives. The *in vivo* efficacy of selected inhibitors will be assessed in a murine macrophage-like cell line, J774A.1, by measuring changes in endogenous levels of host tyrosine phosphorylation associated with the binding and phagocytosis of *Yersinia pseudotuberculosis*. The structural basis of inhibition specificity will be revealed by the determination of the three-dimensional structures of YopH complexed with high affinity inhibitors, which should provide framework for further improvement in inhibitor potency and specificity.

Grant: 1R01AI048829-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: SAMUEL, JAMES E PHD
Title: VACCINE INTERVENTION AGAINST Q FEVER
Institution: TEXAS A&M UNIVERSITY HEALTH SCIENCE COLLEGE STATION, TX
CTR
Project Period: 2000/07/15-2004/06/30

DESCRIPTION: The illegitimate use of a bacterial pathogen is most likely and damaging with an agent that is readily dispersed and infectious by aerosol, stable in the environment after release, and capable of causing panic and debilitation of those affected. *Coxiella burnetii* fulfills all these criteria yet public health response to this agent, including vaccine intervention, is underdeveloped. The agent causes acute Q fever, occasional chronic disease, and very recently was linked to chronic vascular disease. Subunit immunogens that can provide protection against acute and chronic diseases without an adverse response are uncharacterized. The long-range goal of this project is to develop an effective subunit vaccine to provide immunity to individuals that are at risk of infection with *C. burnetii*. The central hypothesis is that outer membrane proteins can be used to develop protective immunity against Q fever. This will be accomplished by pursuing three specific aims. First, several major immunogenic outer membrane antigens will be identified and characterized. Two immunogenic outer membrane antigens, P-1 and Omp47, have been cloned by our group. Additional outer membrane proteins will be evaluated for reactivity with immune sera and major antigens cloned. This will include components of a putative type 4 secretion system. Next, the vaccinogenic potential of immunogenic outer membrane proteins will be evaluated. Mice will be vaccinated with recombinant protein in adjuvant and then challenged with a lethal dose of virulent *C. burnetii*. Alternatively, immunity to selected antigens will be developed by DNA vaccination and evaluated for the ability to protect against lethal challenge. Protective immunogens will also be evaluated in a guinea pig fever model infected through intraperitoneal and aerosol challenges. Finally, the components of the immune response elicited with protective subunit antigens will be characterized. The working hypothesis is that both humoral and cell mediated immune responses will be required to confer protection against a lethal or fever-inducing challenge of *C. burnetii*. The immune profile of protected animals will be evaluated for antibody development, Th1 and Th2 cytokine expression, and T cell responses. Recombinant immunodominant antigens could also be used to develop improved diagnostic tools.

Grant: 1R01AI048945-01
Program Director: LANG, DENNIS R
Principal Investigator: CONWAY, TYRRELL PHD
Title: GROWTH AND COLONIZATION OF THE INTESTINE BY E COLI
Institution: UNIVERSITY OF OKLAHOMA NORMAN NORMAN, OK
Project Period: 2000/06/15-2004/05/31

To colonize an animal host, bacteria must acquire nutrients for growth. However, despite extensive research, next to nothing is known about the nutrients and metabolic pathways used in situ by the 400-500 species which inhabit the mammalian large intestine. The proposed research uses functional genomics technology to investigate the acquisition of nutrients by enteric bacteria during the colonization process. Specifically, we will test the hypothesis that induction of the pathways used for catabolism of mucus-derived sugars is essential for colonization of *E. coli*. Global transcription assays will be used to identify regulons induced for growth on the complex mixture of nutrients present in intestinal mucus. Probes prepared from cells grown on minimal media containing cecal mucus will be hybridized to DNA arrays of all *E. coli* genes. Pathways induced in situ for growth on mucus will be identified by analyzing global transcription patterns of *E. coli* cells in the ceca of experimentally colonized germ-free mice. Pathways which contribute to the ability of *E. coli* to successfully compete with the 400-500 other species in the large intestine will be examined. Mutant strains selectively blocked in specific catabolic pathways will be tested for their ability to colonize mice and green fluorescent protein reporter fusions will be used to determine the temporal and spatial expression of these regulons in individual bacterial cells during colonization. These experiments will identify the distinct physiological roles of individual mucosal sugars in colonization of the large intestine by *E. coli*, providing knowledge important for addressing the more general question of whether or not metabolic diversity allows pathogenic *E. coli* strains to colonize in the presence of precolonized strains, thus leading to their ability to cause disease. Knowing precisely which nutrients are essential for colonization should lead to improved strategies for combating infection.

Grant: 1R01AI049174-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: FAHEY, ROBERT C BS
Title: MYCOTHIOIOL BIOSYNTHESIS AND METABOLISM AS TB DRUG TARGETS
Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA
Project Period: 2000/08/01-2003/07/31

Abstract Text Not Available

Grant: 1R01AI049740-01
Program Director: LANG, DENNIS R
Principal Investigator: LEONG, JOHN M MD
Title: ROLE OF INTIMIN IN TISSUE TROPISM AND DAMAGE BY EHEC
Institution: UNIV OF MASSACHUSETTS MED SCH WORCESTER, MA
WORCESTER
Project Period: 2000/09/30-2004/09/29

DESCRIPTION (adapted from the application) Enterohemorrhagic E. coli (EHEC) is an important cause of diarrheal disease and renal failure in the U.S. EHEC colonizes the mucosa of the large bowel, and the extraintestinal manifestations of EHEC infection result from the absorption across the epithelium of Shiga-like toxin (Stx) produced by intestinal EHEC. During attachment to colonic epithelium, the bacterium disrupts the host cell cytoskeleton and forms a highly organized cytoskeletal structure underneath the bound bacterium, termed an attaching and effacing (AE) lesion. Intimin, a bacterial outer membrane protein that mediates tight host cell attachment, is required for AE lesion formation and full virulence. Experimental infection with EHEC expressing intimin from enteropathogenic E. coli (EPEC), a pathogen that infects a different intestinal site, suggested that intimin influences tissue tropism. We postulate that: (1) intimin plays a central role in determining the site of colonization; and that (2) intimin-mediated cytoskeletal disruption of intestinal epithelial cells facilitates delivery of Stx to extraintestinal sites. To characterize the features of intimin that influence tissue tropism and promote Stx translocation, the following questions will be addressed: 1. Does an EHEC strain that expresses EPEC intimin under appropriate regulatory controls demonstrate altered tissue tropism? The EHEC chromosomal eae coding sequence will be specifically replaced by the EPEC eae coding sequence, and the effect of this alteration on tissue tropism will be assessed. 2. What domain of intimin influences the site of intestinal colonization? The region of EPEC intimin responsible for the differences in tissue tropism that we expect to find in Aim 1 will be identified by analyzing strains that express hybrid EHEC/EPEC intimin proteins. 3. Does the ability to generate robust AE lesions correlate with mucosal damage and/or toxin translocation? Isogenic EHEC strains differing in their ability to generate AE lesions will be characterized for differences in mucosal damage and in translocation of toxin across intestinal epithelium. By developing a detailed understanding of the role of intimin in the pathogenesis of EHEC, the proposed experiments may lead to therapeutic strategies designed to prevent colonization at one of the earliest steps or to minimize the intestinal absorption of toxin after infection has been established.

Grant: 1R01AI049741-01
Program Director: LANG, DENNIS R
Principal Investigator: NEISH, ANDREW S MD
Title: BACTERIAL MODULATION OF EPITHELIAL SIGNALING PATHWAYS
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 2000/09/30-2004/08/31

DESCRIPTION (adapted from the application) Salmonella typhimurium is a common cause of food-borne enterocolitis in this country and the developing world. The clinical manifestations of Salmonella typhimurium infection result from inflammatory cells that are recruited to, and accumulate in, the intestinal mucosa. The intestinal inflammatory response is mediated in large part by synthetic upregulation of secreted cytokines and other inflammatory effector molecules. These inflammatory mediators are activated at the transcriptional level by the action of DNA binding transcription factors such as NF-kappaB. Experiments with cultured intestinal epithelial cells have shown that S. typhimurim associated with inflammatory intestinal disease activate NF-kappaB via a calcium dependant activation pathway, while other, non-pathogenic Salmonella strains repress activation of this key regulator. Our overall hypothesis is that Salmonella (and potentially other enteric bacteria) have evolved novel mechanisms to modulate epithelial signaling pathways that may serve to establish either a pathologic or a commensal state. Elucidation of these influences will increase our understanding of the epithelial and bacterial factors involved in human enterocolitis, as well as the microbiology of this class of enteric infections. This application describes a variety of experimental methods to study the host inflammatory response including pathogen induced calcium dependant activation of the NF-kappaB pathway. These methods will be applied to signals elicited by S. typhimurium, a series of Salmonella mutants and variety of other enteric pathogens. Secondly, similar assays will be used to study the mechanism by which some bacteria repress host immune and inflammatory reactions by modulation of the NF-kappaB signaling cascade. These studies will be supported using well characterized murine models of intestinal inflammation to correlate the anti-inflammatory properties of these bacteria in vivo. Finally, a novel electroporation based method to introduce purified proteins into intact model epithelia will be used to dissect the cellular responses to internalization of a variety of bacterial effector proteins.

Grant: 1R01AI049842-01
Program Director: LANG, DENNIS R
Principal Investigator: SCHAUER, DAVID B PHD
Title: O157:H7 INTIMIN AND STX--PATHOGENESIS IN A MOUSE MODEL
Institution: MASSACHUSETTS INSTITUTE OF CAMBRIDGE, MA
TECHNOLOGY
Project Period: 2000/09/30-2004/09/29

DESCRIPTION (adapted from the application) Since the early 1980s, enterohemorrhagic *Escherichia coli* (EHEC) has been recognized as a cause of diarrhea and hemorrhagic colitis. The most serious complication of EHEC infection is hemolytic uremic syndrome (HUS), which occurs primarily in children. While it is now recognized that EHEC strains belonging to a variety of serotypes can cause HUS, O157:H7 is the dominant EHEC serotype in many parts of the world and has been the type most commonly associated with foodborne outbreaks. EHEC O157:H7 virulence determinants include shiga toxins (Stx), a chromosomal pathogenicity island called the locus for enterocyte effacement (LEE) that encodes the adhesin intimin and mediates attaching and effacing (AE) activity, as well as a hemolysin (HlyA), a serine protease (EspP), and an plasmid-encoded adhesin with homology to the *Clostridium difficile* toxin B (Efa1). We have developed a mouse model of *Citrobacter rodentium* colitis that is dependent on LEE-encoded gene products and AE activity for colonization. In this model, suckling mice develop significant mucosal damage in the colon, which is associated with severe colitis and death. Adult mice, on the other hand, develop colonic epithelial hyperplastic and a milder colitis that is typically subclinical. We have recently discovered that an increased inoculum of *C. rodentium* (10⁹ CFU, intragastric) leads to significant mucosal damage and severe colitis in adult mice. We now propose to develop a mouse model of AE colitis for O157:H7 by introducing individual virulence determinants from EHEC O157:H7 into *C. rodentium* and using these strains to infect adult mice. Specifically, we will 1) characterize the role of O157: H7 intimin in this mouse model of AE colitis, 2) characterize the role of Stx in this mouse model of AE colitis, and 3) characterize the mucosal response to O157:H7 intimin and to Stx in vitro and in vivo. Overall, this work will allow us to test the hypothesis that AE-dependent mucosal damage and colitis influence Stx translocation from the gut. It should also lead to the development of a mouse model for studies of O157:H7 pathogenesis and for testing preventive and therapeutic approaches directed against this important foodborne illness.

Grant: 1R03AI047045-01
Program Director: LAUGHON, BARBARA E.
Principal Investigator: MADIRAJU, MURTY V
Title: MYCOBACTERIUM AVIUM DNA B AND NOVEL DRUG TARGET
Institution: UNIVERSITY OF TEXAS HLTH CTR AT TYLER, TX
TYLER
Project Period: 2000/06/01-2003/05/31

DESCRIPTION: (Adapted from Applicant's Abstract) MACs are the most common cause of mycobacterial lung disease other than tuberculosis and are the leading cause of morbidity and mortality in HIV-infected AIDS patients. At present the antibiotics available for treating M.avium infections are inadequate. DNA replication leading to multiplication of a pathogen is the first committed step in the onset of infection. Replication is believed to be regulated at the level of initiation. The present proposal focuses on the DnaB protein, whose analogs in other bacteria play a crucial role in replication initiation and the DnaB protein is essential for cellular growth. The interactions of the DnaB protein with the complexes of the origin of replication and DnaA, the putative initiator protein believed to mark the future forks for bidirectional replication. The DnaB protein of M.avium, unlike many other bacterial DnaB, contains Intein in its sequence. Experiments proposed here will examine the roles of DnaB and Intein proteins, nature and functional consequences of Intein splicing in M.avium DNA replication and metabolism. The long term goal is to be able to develop drugs that will block the initiation process and thereby prevent growth and development of M.avium infections.

Grant: 1R03AI048492-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: BENACH, JORGE L PHD
MICROBIOLOGY: BACTERIOLOGY
OGY
Title: HOST RESPONSES TO THE TULAREMIA AGENT
Institution: STATE UNIVERSITY NEW YORK STONY BROOK STONY BROOK, NY
Project Period: 2000/08/01-2003/07/31

Human tularemia is a highly virulent bacterial zoonosis with endemic foci in the northern hemisphere. Its clinical manifestations depend on the route of infection. The ulceroglandular form is the most common presentation after handling sources contaminated with *Francisella tularensis*. When ingested, contaminated food or water can cause an oropharyngeal form. Pulmonary, typhoidal (the two more common forms reported in laboratory workers), glandular and ocular forms are other less frequent presentations. The disease occurs in outbreaks, usually associated with direct contact with infected game or contaminated water, or in a seasonal pattern in arthropod-borne tularemia. The severity of this infection, its initial nonspecific manifestations, and the ability of the agent to survive in the environment have led to the inclusion of *F. tularensis* in a list of bacterial pathogens that could be used for bioterrorism. The human and murine responses to *F. tularensis* have been studied with particular emphasis on the survival of the bacterium within macrophages and the cytokine responses resulting from intracellular infection. Less known are the interactions of this organism with cells that it must encounter to cause systemic infection. Thus, a very focused study of the interaction of *F. tularensis* with endothelium is proposed for this R03 application. Both the attenuated vaccine strain and the virulent American strain (*F. tularensis tularensis*) will be used for parallel experiments on infection of endothelium and its pro-inflammatory activation, as measured by upregulation of expression of adhesion molecules and chemokines. One feature of tularemia that has not been investigated is the manner of spread of this organism within the infected host. Tularemic lesions contain a marked mononuclear cell infiltrate, and the sequence of lesion formation is not known. Trafficking of the bacterium alone and of cells infected with the bacterium (neutrophils, monocytes) will be studied by assessing their ability to cross endothelium in vitro. Earlier studies have found that the lipopolysaccharide of *F. tularensis* does not activate mononuclear cells, so its effect on stimulation of endothelial cells is not clear, nor is there a certainty that there is a CD14-dependency of the response to the lipopolysaccharide or the whole organism. The proposed research is aimed at a greater understanding of the mechanisms used by *F. tularensis* to cause systemic infection.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R03AI049161-01
Program Director: LANG, DENNIS R
Principal Investigator: BERG, DOUGLAS E PHD
Title: Molecular Genetics of H. Pylori In India
Institution: WASHINGTON UNIVERSITY ST LOUIS, MO
Project Period: 2000/09/01-2003/01/31

Abstract Text Not Available

Grant: 2R15AI037773-02
Program Director: KLEIN, DAVID L
Principal Investigator: TEMPLE, LOUISE M PHD
Title: CHARACTERIZATION OF BORDETELLA AVIUM ATTACHMENT FACTORS
Institution: DREW UNIVERSITY MADISON, NJ
Project Period: 1997/04/01-2003/04/30

The broad based pathogenesis study described here accomplishes all of the AREA program objectives: (1) supporting meritorious research, (2) exposing undergraduates to research, and (3) strengthening the research environments in non-research intensive schools. The experimental model, *Bordetella avium*, causes a highly contagious respiratory disease in its avian hosts, primarily turkeys, with striking similarity to whooping cough in children, caused by *B. pertussis*. Species specificity is determined largely by the attachment process of the bacteria to the tracheal cilia of the relevant host. The proposed study focuses on the attachment process. The specific aims are to identify and characterize the specific bacterial molecules involved in attachment. Using both genetic and biochemical approaches, this study began with several well-defined mutant strains of *B. avium* which had lowered attachment rates in vivo and in vitro. Several mutants mapped to a *B. avium* chromosome region encoding homologs of *B. pertussis* pili and filamentous hemagglutinin, molecules identified in the attachment process in *B. pertussis*. These molecules will be purified and characterized, and other molecules essential to attachment will be identified and studied using two models, tracheal ring attachment and hemagglutination of guinea pig erythrocytes. With this methodical approach of creating and analyzing relevant mutants and biochemically characterizing attachment molecules, the attachment process will be elucidated and described at the molecular level. The systematic examination of virulence factors in *B. avium* will yield important and useful information about that organism and the disease in turkeys, and may prove helpful in understanding disease causation among all the *Bordetella* species. Over the 3 year funding period, this project will expose more than 60 students in laboratory and independent research to an excellent model of research and give them an opportunity to make unique contributions to the study of an important pathogen. Having a broad based approach provides students with numerous opportunities to explore relevant scientific questions, exert creative thinking, and learn the fundamentals of biomedical research. The ongoing collaborative study has served a catalytic role over the last four years, increasing the interest of students and faculty alike in research, and will continue to enhance the research environment for undergraduates in the sciences at Drew University.

Grant: 1R15AI044776-01A1
Program Director: KLEIN, DAVID L
Principal Investigator: ACTIS, LUIS A PHD BIOCHEMISTRY
Title: GENETICS OF H. AEGYPTIUS BRAZILIAN PURPURIC FEVER CLONE
Institution: MIAMI UNIVERSITY OXFORD OXFORD, OH
Project Period: 2000/02/15-2004/01/31

Brazilian purpuric fever (BPF) is a fatal disease caused by a unique invasive clone of *Haemophilus influenzae* biogroup *aegyptius* (*H. aegyptius*), a bacterial species that is normally non-invasive and causes mild cases of conjunctivitis. The unusual invasive attributes of the BPF clone of *H. aegyptius* make this pathogen an ideal model to study the emergence of an invasive derivative, which expresses new virulence determinants, from an old bacterial pathogen. Furthermore, the availability of a tissue culture model using the natural host-target cells and the existence interactions, bacterial gene transfer, and the regulation of the expression of bacterial virulence genes. In addition, little is known about the molecular and genetic nature of the BPF virulence factors and the pathogenesis of the purpura fulminans caused by this pathogen. Thus, the long-term goal of this research project is the elucidation of the genetic and molecular mechanisms involved in the invasion and destruction of the endothelial host-cells by the BPF clone. In this proposal, we address this overall goal through several approaches, combining classical methods used in bacterial genetics with techniques designed to isolate unique genes present in virulent strains and examine differential gene expression. The first Specific Aim involves the isolation and characterization of unique BPF genes, some of which may be involved in virulence. This will be achieved by constructing a gene library using the innovative subtraction genomic hybridization technique. The second Specific Aim is focused on the expression analysis of the unique BPF genes at the transcriptional and translational levels using northern and western blotting together with reverse transcriptase- polymerase chain reaction. The third Specific Aim examines the role of unique BPF genes in the invasion and destruction of human endothelial cells using isogenic mutants. These mutants, which will be generated by site-directed insertion mutagenesis and allelic exchange, will be tested in the HMEC-1 tissue culture model that mimics the vascular destruction produced during the infection process caused by the invasive strains of *H. aegyptius*. These proposed studies address an important and largely unexplored aspect of the pathogenesis of the vascular destruction caused by the BPF clone of *H. aegyptius*. Furthermore, these studies will lead to a better comprehension of the nature of other vascular destructive infectious diseases.

Grant: 1R15AI045522-01A1
Program Director: LANG, DENNIS R
Principal Investigator: LOGUE, CATHERINE M
Title: ANTIBODIES TO ISS FOR STUDY OF VIRULENT E. COLI
Institution: NORTH DAKOTA STATE UNIVERSITY FARGO, ND
Project Period: 2000/07/01-2004/06/30

DESCRIPTION (Adapted from the applicant's abstract): Colibacillosis is a serious economical problem for the animal production industry. In addition, it is a serious concern for human health because animals are an important source of Escherichia coli strains that can cause severe infections in humans. Data from the PI's laboratory suggest that complement resistance is an important bacterial virulence factor that contributes to the pathogenicity of extraintestinal infections in birds, which are caused by avian isolates of E. coli. This project is design to isolate and produce monoclonal antibodies against Iss, a plasmid-encoded outer membrane protein that contributes to complement resistance by a mechanism that remains to be elucidated. The antibodies will be used to confirm the surface exposure of Iss and examine if the amount of surface-exposed Iss is correlated with the virulence and complement resistance of E. coli avian isolates. This project will be used to introduce and train undergraduate students and strengthen the research program at North Dakota State University.

Grant: 1R15AI046481-01
Program Director: LANG, DENNIS R
Principal Investigator: WILMES-RIESENBERG, MARY R PHD
Title: IDENTIFICATION OF REGULATORS OF SALMONELLA INVASION
Institution: BOWLING GREEN STATE UNIV BOWLING BOWLING GREEN, OH
GREEN
Project Period: 2000/03/01-2001/08/15

Salmonellosis represents a serious global problem. The incidence of infection continues to increase worldwide, despite expensive research and changes at the production and processing levels. Our long term goal is to control Salmonella infection in humans and animals and to develop Salmonella-based vaccines to deliver heterologous antigens. Our objective, as outlined in this proposal, is to elucidate the genetic regulation of Salmonella genes whose products are required invasion. When Salmonella bacteria invade a host cell, they secrete effector proteins via a type III secretion system that are translocated into the host cell. Importantly, this secretion system has been shown to be an effective delivery system of viral epitopes to the host cell cytosol, hence it is being developed for use in vaccines against viral antigens and tumor-specific antigens. Understanding the regulation of the genes involved in type III secretion will enable us to design efficacious vaccine strains which will optimally deliver heterologous antigens. Specifically we will (i) identify genes which encode proteins regulating the *invA* gene (invasion regulator; *inr* genes), (ii) determine if the *Inr* proteins regulation other invasion genes encoded in the Salmonella pathogenicity island I, and (iii) identify other genes regulated by *Inr* and determine if they are required for virulence of Salmonella. Our approach will involve using a variety of genetic and molecular biology techniques to identify mutants that show an altered expression of the invasion genes. These mutants will be used to identify additional virulence genes in Salmonella. Mutants generated in this study will be evaluated using tissue cultured and animal models.

Grant: 1R15AI046493-01A1
Program Director: MILLER, MARISSA A.
Principal Investigator: PAPASIAN, CHRISTOPHER J PHD
Title: CONTRIBUTION OF LPS TO EXP ENTEROCOCCAL SEPSIS
Institution: UNIVERSITY OF MISSOURI KANSAS CITY KANSAS CITY, MO
Project Period: 2000/07/01-2003/06/30

DESCRIPTION (Adapted from the Applicant's Abstract): Severe sepsis and septic shock secondary to Gram-positive bacterial pathogen infection is increasingly recognized in a recent series of patients with sepsis from the USA and other developed countries. While the molecular pathogenesis of Gram-negative bacterial sepsis and endotoxin-mediated cellular events are increasingly well understood, the molecular nature of microbial pathogenesis secondary to Gram-positive bacterial pathogens remains less well understood. Superantigen-mediated immunopathogenic mechanisms are well described but the pathogenesis of non-superantigen-producing Gram-positive pathogens is less well understood. A major bacterial pathogen in recent studies of septic shock has been the enterococcal species. These Gram-positive bacterial organisms are intrinsically resistant to numerous antimicrobial agents and have a propensity for rapid development of resistance to broad-spectrum antibacterial agents including vancomycin, aminoglycosides, and extended spectrum beta-lactam antimicrobial agents. It has been previously observed that enterococcal species, in contrast to other Gram-positive bacterial pathogens, are more likely to cause lethality in D-galactosamine-treated mice. D-galactosamine treatment potentiates TNF-mediated lethality in this rodent model. These data indicate that TNF is important in the lethality associated with enterococcal sepsis. Further support for this hypothesis is found in corticosteroid-treated animals. Corticosteroids attenuate TNF-induced lethality in gram-negative bacterial sepsis models. This steroid-induced attenuation is observed with enterococci but not other Gram-positive bacterial pathogens tested thus far. It was observed that *Enterococcus faecalis* was a poor inducer of TNF in an in vitro system that examines TNF production in peritoneal macrophages. The investigators hypothesize that Enterococcal species must synergize with bacterial LPS in Gram-negative bacteria in order to induce a lethal systemic inflammatory response. The proposal will investigate the general nature of enterococcal sepsis through a series of experiments. First, the investigators plan to test a wider array of Gram-positive pathogens including other species of enterococci to determine the potential unique position of *Enterococcus* species in the generation of a lethal systemic bacterial infection. Next the investigators will use an anti-TNF monoclonal antibody as well as a TNF knockout mouse in addition to an LPS hyporesponsive mouse model and a lipopolyamine anti-endotoxin agent in combination with enterococcal bacterial challenge to examine the relative roles of TNF and endotoxin in enterococcal sepsis.

Grant: 1R15AI046506-01
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: RUST, LYNN PHD
Title: PSEUDOMONAS AERUGINOSA LASB PROMOTER INTERACTIONS
Institution: NORTH DAKOTA STATE UNIVERSITY FARGO, ND
Project Period: 2000/03/01-2003/02/28

Elastase, coded by the *lasB* gene, is an important virulence determinant of *Pseudomonas aeruginosa*. Development of a model of *lasB* induction is essential to understanding the global induction of virulence factors which contributes to morbidity and mortality from acute and chronic *P. aeruginosa* infections. *P. aeruginosa* uses a bacterial cell density-dependent ("quorum sensing") system of gene activation to induce three protease structural genes, including *lasB*. This activation is mediated by the complex of a regulatory protein (LasR) and an N-acylhomoserine lactone, or autoinducer (PAI-1). Two operators are present in the regulatory region of *lasB*, one of which (OP1) is responsive to the LasR/PAI-1 complex. Preliminary data suggest that the two operators function synergistically to activate *lasB*, and that the second operator (OP2) is a minor OP I-like operator. Gel shift and DNase I footprinting assays will be used to elucidate the roles of LasR/PAI-1, OP1, and OP2 in *lasB* activation. The experiments outlined will determine whether *lasB* is a class II promoter, and whether the function of OP2 is to facilitate cooperative protein-protein binding. Specific aims are: 1) To determine the function of OP1 in *lasB* activation: a) Does LasR bind OP1?, b) Does LasR interact with RNAP alphaCTD in a class II-fashion?, and, c) Does LasR directly facilitate isomerization? 2) To determine the function of OP2 in *lasB* activation: a) Does LasR bind OP2?, b) Do LasR at OP2 and RNAP bind cooperatively?, and, c) Does LasR bind OP1 and OP2 cooperatively?

Grant: 1R15AI047115-01
Program Director: MILLER, MARISSA A.
Principal Investigator: TOLMASKY, MARCELO E PHD
Title: DEALING WITH ANTIBIOTIC RESISTANCE--ANTISENSE TECHNOLOGY
Institution: CALIFORNIA STATE UNIVERSITY FULLERTON, CA
FULLERTON
Project Period: 2000/06/01-2005/05/31

DESCRIPTION (Adapted from Applicant's Abstract): Drug resistance is a major obstacle in the conquest of bacterial infections. This proposal targets a critical issue in the treatment of bacterial infectious diseases: the need to develop strategies aimed at preserving the effectiveness of currently available aminoglycoside antibiotics. The investigators focus on resistance to the aminoglycoside amikacin mediated by the aminoglycoside 6'-N-acetyltransferase AAC(6')-Ib, which can also modify several other aminoglycoside antibiotics. The long term goal of this research project is to develop the use of antisense oligonucleotides as tools to selectively inhibit the expression of aac(6')-Ib and other related genes. The specific aims for this grant proposal are: 1a) Identification of synthetic oligonucleotides and analogs that promote cleavage of aac(6')-Ib mRNA by RNase H or RNase P. 1b) Bioavailability studies of synthetic oligonucleotides and oligonucleotide analogues. RNase H or RNase P can mediate inhibition of gene expression by degradation of mRNA in the presence of appropriate antisense molecules. The general strategy of this specific aim will consist of the utilization of two conceptually different approaches to identify regions in the mRNA available for interaction with oligonucleotides. With this information, oligodeoxynucleotides and analogues will be designed to inhibit the expression of aac(6')-Ib by inducing RNase H degradation of the mRNA. The information will also be utilized for the development of antisense oligoribonucleotides that inhibit the expression of aac(6')-Ib by inducing RNase P degradation of the mRNA. Following, the investigators will initiate studies on the cell uptake of oligonucleotides and analogues. 2a) Development of peptide nucleic acid (PNA) molecules that inhibit expression of AAC(6')-Ib. 2b) Bioavailability studies of PNA molecules. A novel strategy to inhibit gene expression using antisense technology is now available with the synthesis of peptide nucleic acids (PNAs). PNAs may be developed as antimicrobial agents in prokaryotic systems. The investigators will test the in vitro and in vivo activity of several PNA molecules to interfere with the expression of aac(6')-Ib. The investigators will then study the bioavailability of naked and liposome encapsulated PNA molecules.

Grant: 1R15AI047165-01
Program Director: TSENG, CHRISTOPHER K.
Principal Investigator: BLAZYK, JOHN F PHD
Title: DESIGN OF NOVEL LINEAR CATIONIC ANTIMICROBIAL PEPTIDES
Institution: OHIO UNIVERSITY ATHENS ATHENS, OH
Project Period: 2000/06/01-2002/07/14

DESCRIPTION (adapted from applicant's abstract): Antibiotics have proven effective in eliminating or at least greatly reducing the incidence of many diseases caused by bacteria because these compounds possess the necessary selectivity to attack bacterial cells while sparing human cells. Unfortunately, the widespread use of common antibiotics such as penicillin has selected for resistant strains that are no longer susceptible to these agents. Since resistance is appearing to even the most potent antibiotics, such as vancomycin, the development of new approaches in antimicrobial therapy is imperative. The discovery of naturally occurring antimicrobial peptides opened a new dimension for antibiotic development. Magainins, isolated from frog skin, are representative of the class of small linear cationic peptides that can kill both Gram-positive and Gram-negative bacteria by increasing the permeability of the plasma membrane at concentrations that do not induce hemolysis. PGLa, also isolated from frog skin, has greater antimicrobial activity than magainins while retaining low hemolytic activity. A common feature of these peptides is their capacity to form an amphipathic alpha-helix (with polar and nonpolar groups on opposite faces of the helix), a structural feature believed to be important in their function as antimicrobial agents. Numerous analogues with sequences derived from these peptides have been prepared and examined. In nearly all cases, the strategy employed in enhancing activity involved increasing the amphi-pathic alpha-helical character of the peptide. To date, no one has attempted a comprehensive study of the structure-function relationships of a family of closely related linear peptides with simple sequences designed to adopt different secondary structures. This approach will reveal the importance of different secondary structures with varying levels of amphipathic character in determining antimicrobial activity and selectivity between bacterial and mammalian membranes. We propose here to synthesize two families of peptides with varying capacity to form amphipathic alpha-helic and beta-sheet structures. Using these two families of peptides, we will investigate the following areas: 1) the relationship of secondary structure and amphipathic character of the peptides to antimicrobial activity; 2) the amount of peptide that must bind to the membrane in order to increase permeability; and 3) the role of the membrane lipid composition in determining susceptibility to peptide-induced increase in permeability. This project should facilitate the design of effective antimicrobial peptides that will augment the arsenal of available antibiotics in order to keep pace with the ever-increasing resistance of bacteria to the drugs in current use.

Grant: 1R15AI047297-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: ENNIS, D G PHD
Title: ANALYSIS OF DNA REPAIR AND SOS REGULATION IN BRUCELLA
Institution: UNIVERSITY OF LOUISIANA AT LAFAYETTE LAFAYETTE, LA
Project Period: 2000/03/01-2003/08/30

Our principal goal is to analyze the role(s) that DNA repair and recombination functions play in relevant models of bacterial pathogenesis. In selected bacterial systems, DNA repair functions are known to contribute to pathogenesis. For example, repair-defective mutants of Salmonella were found to lose pathogenicity; and the loss of virulence was correlated with the inability of these mutants to survive the damage from the oxidative burst within the macrophage. A number of pathogens not only survive, but even flourish within professional phagocytes; these include some Brucellaeae, Campylobacteraeae, Chlamydiaeae, Edwardsiellaeae, Listeriaeae, Mycobacterieaeae and Yersineae. The function the repair mechanisms play in virulence of each of these pathogens is not yet understood. Our major emphasis will be to study DNA repair in certain representative bacteria with a focus Brucella abortus, which causes undulant fever in humans and abortions and infertility in cattle and other domestic animals. Little is understood about DNA repair in Brucella abortus; a better characterization of repair in these organisms will be required to determine its role(s) in virulence. We propose the following specific aims to address the question "What are the roles of DNA repair and recombination in pathogenesis of specific intracellular bacteria?": 1) Isolation and molecular characterization of specific repair and recombination genes in Brucella abortus, 2) Characterize the SOS regulation of DNA repair genes in Brucella. 3.) Analysis of inhibition of the Brucella abortus repair networks by dominant defective recA mutants.

Grant: 1R15AI047412-01
Program Director: LANG, DENNIS R
Principal Investigator: PISTOLE, THOMAS G PHD
Title: HOST CELL RECOGNITION OF SALMONELLA TYPHIMURIUM
Institution: UNIVERSITY OF NEW HAMPSHIRE DURHAM, NH
Project Period: 2000/05/01-2003/04/30

DESCRIPTION (ADAPTED FROM THE APPLICANT'S ABSTRACT): 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 interaction of salmonellae with human-derived cells: intestinal epithelial cells and macrophages. The first objective is to determine the role of the porins OmpC and OmpD in mediating attachment of *S. typhimurium* to and internalization by these host cells. In these studies mutants deficient in one or both porin genes will be compared to the parental wild type strain in their ability to adhere to and become internalized by target host cells. Bacteria-eukaryotic association will be measured by fluorescence microscopy and flow cytometry. The second objective is to determine the ability of porins or porin-lipopolysaccharide complexes to inhibit bacterial attachment to host cells. Partially purified porins, containing associated lipopolysaccharide, and highly purified porins will be compared for the respective ability to block attachment, again using fluorescence microscopy and flow cytometry. The third objective will use non-targeted approaches to identify major bacterial ligands involved in interactions with target human cells. Potential molecules will be identified by probing bacterial envelope proteins with labeled host cells and by recovering Salmonella-derived binding protein by elution from host cells. Antiserum generated against these proteins will be used in binding inhibition studies. These studies are expected to provide a better understanding of the early cellular events in Salmonella infections. Such information may prove useful in developing microbial vaccines both for homologous protection and for hybrid immunogens that use Salmonella as a vector.

Grant: 1R15AI047413-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: LITTLE, SUSAN E DVM
Title: GOAT MODEL OF EHRLICHIA CHAFFEENSIS RESERVOIR INFECTION
Institution: UNIVERSITY OF GEORGIA ATHENS, GA
Project Period: 2000/04/01-2004/03/31

Our long range goal is to understand how *E. chaffeensis* and other ehrlichial organisms are maintained in nature. As a major objective in pursuit of this goal, this application proposes to develop the domestic goat as a model of *E. chaffeensis* vertebrate infection. The central hypothesis is that domestic goats can serve as a much-needed surrogate to white-tailed deer in laboratory based studies of *E. chaffeensis* reservoir infection dynamics. The rationale behind the proposed research is that goats could provide a superior laboratory model of reservoir host infection to white-tailed deer. Goats have a much more tractable nature than deer and they remain easy to handle throughout their life. This docile nature allows greater latitude in the design of infection studies and reduces the risk of handling-associated injury. In addition, goats are more widely available than deer for research, a higher degree of similarity can be obtained between experimental animals due to controlled inbreeding, they are easily raised in tick-free (and thus Ehrlichia-exposure free) facilities, and a larger variety of laboratory reagents are available for work with goats. In short, a goat model of vertebrate reservoir infection, once developed, would allow design and conduct of experiments not readily possible with deer. To accomplish the objective of this application, we will pursue four specific aims: (1) determine how *E. chaffeensis* infection develops and is maintained in domestic goats, (2) evaluate the persistence of *E. chaffeensis* infection in the absence of re-exposure, domestic goats, (2) evaluate the persistence of *E. chaffeensis* infection in the absence of re-exposure, (3) evaluate the response of goats to multiple re-challenge with a homologous strain of *E. chaffeensis*, and (4) transmit *E. chaffeensis* from infected to naive domestic goats via tick feeding. At the completion of this research we expect to have developed an alternative ruminant model for studying *E. chaffeensis* within the vertebrate reservoir host by characterizing the course of *E. chaffeensis* infection within the domestic goat. Additionally, we will have evaluated both the persistence of infection in goats in the absence of re-exposure and the response of goats to multiple, frequent re-exposure, as well as have developed a natural model of infection in goats via tick feeding. The development of this model and its subsequent application in future experiments will facilitate understanding about the dynamics of *E. chaffeensis* infection in the natural wild reservoir host. New control strategies could result.

Grant: 1R15AI047795-01
Program Director: FAIRFIELD, ALEXANDRA
Principal Investigator: JARROLL, EDWARD L PHD
Title: GIARDIA ENCYSTMENT: CONTROL OF GALNAC SYNTHESIS
Institution: NORTHEASTERN UNIVERSITY BOSTON, MA
Project Period: 2000/08/15-2004/08/14

Giardia causes is a major intestinal illness in the United States as well as many other countries worldwide. The life cycle is simple and direct requiring a vegetative trophozoite which attaches to the microvillus brush border of the host's intestine and a cyst which passes from host to host by the fecal-oral route. During cyst formation (encystment) induced by bile, Giardia trophozoites form a protective cyst wall filament rich in the cyst wall specific sugar N-acetylgalactosamine (GalNAC, as a novel [GalNAC beta1 yields 3 GalNAC]n homopolymer). GalNAC, undetected in non-encysting trophozoites, is synthesized from glucose during encystment by the activity of five inducible, nonsedimentable enzymes: Glucosamine 6-phosphate isomerase, GPI, glucosamine 6-phosphate N-acetylase (GlcNPA), phosphoacetylglucosamine mutase (PAG1cNM), UDP-N-acetylglucosamine pyrophosphorylase (UDP-GlcNAcPP), and UDP-N-acetylglucosamine 4' epimerase (UDP-GlcNAcE). The goal of our laboratories is an in-depth study of enzyme regulation for, the molecular biology for the control of, and the possible development of new chemotherapeutic agents that can target this pathway thus acting to prevent the formation of cysts and thus aiding in the control of giardiasis. To date GPI, UDP-GlcNAcPP and CWS have been purified (or partially purified) and characterized, and GPI and UDP-GlcNAcPP have been cloned and sequenced. We have shown also that GPI is transcriptionally regulated while UDP-GlcNAcPP is constitutive but unidirectionally activated toward GalNAC synthesis by glucosamine 6-PO4 the anabolic product of GPI. Before more in depth studies of the regulation of this pathway can be undertaken, it is essential to understand more about the three enzymes which have not yet been purified or characterized. Thus, we plan to use molecular techniques coupled with enzyme analyses to 1) determine whether GlcNPA, PAG1cNM, and UDP-GlcNAcE activities are induced at a transcriptional level (as is the case with GPI) or at a post-transcriptional level (as is the case with Giardia's UDP-N-acetylglucosamine pyrophosphorylase), and 2) determine if GlcNPA, PAG1cNM, and UDP-GlcNAcE are regulatory enzymes in the GalNAC synthetic pathway by purifying (or expressing) these enzymes and characterizing them with respect to enzyme kinetics, and possible activators and inhibitors.

Grant: 1R15AI047854-01
Program Director: MILLER, MARISSA A.
Principal Investigator: MEADE, MAURA J PHD
Title: NOVEL TRICLOSAN RESISTANCE MECHANISM IN BACTERIA
Institution: ALLEGHENY COLLEGE MEADVILLE, PA
Project Period: 2000/07/01-2003/06/30

Triclosan is a broad spectrum antimicrobial agent that functions by inhibiting fatty acid biosynthesis. It is incorporated into hospital and household antiseptic products alike. Previous research unveiled two separate mechanisms of bacterial resistance to the antimicrobial agent -- modification of the drug site of action and active efflux from exposed cells. Both mechanisms, however, conferred resistance to triclosan concentrations lower than those used in antiseptic formulations. The PI has recently isolated three different microorganisms, including a fluorescent *Pseudomonas* sp. strain called TriR, that are resistant to triclosan at concentrations equivalent to those of antiseptic formulations. The primary research objective is to determine the resistance mechanism of these organisms, which is hypothesized to function through degradation of triclosan. When plated on triclosan-containing media, these organisms produce a clearing zone around the colonies. High pressure liquid chromatography will be utilized to determine if the triclosan concentration decreases over time in these cultures. It is also important to determine if triclosan resistance is plasmid-borne and therefore transferable. This will be accomplished through plasmid curing and triparental mating experiments between the resistant organisms and susceptible bacterial such as *Escherichia coli*. Finally the gene responsible for triclosan resistance in *Pseudomonas* sp. TriR will be isolated. This will be accomplished by transforming susceptible *E. coli* with a *Pseudomonas* sp. TriR cosmid library with subsequent selection on triclosan-containing medium. Since it is unlikely that a modification of fatty acid biosynthesis or active efflux of triclosan would result in the clearing of the suspended antibiotic from bacterial growth media, the PI expects to uncover a new triclosan resistance mechanism. The resistance determinant isolated from TriR will then be sought in the other isolates through hybridization analysis. The results of these proposed will provide valuable information on a novel mechanism of resistance to the antimicrobial agent triclosan at commercially significant concentrations and will provide a basis for future molecular and biochemical studies.

Grant: 1R15AI048397-01
Program Director: LAUGHON, BARBARA E.
Principal Investigator: HEARN, MICHAEL PHD
Title: NEW AGENTS FOR AIDS-ASSOCIATED MYCOBACTERIAL INFECTIONS
Institution: WELLESLEY COLLEGE WELLESLEY, MA
Project Period: 2000/08/01-2003/07/31

DESCRIPTION:(Adapted from the Applicant's abstract) The purpose of this proposal is the discovery of novel agents for the treatment of AIDS-associated opportunistic mycobacterial infections. The hypothesis is that new drugs can achieve improved entry into mycobacteria and enhanced activity against these pathogens by augmentation of drug lipophilicity. The proposal has three specific aims: 1) Prepare novel agents using methods of synthetic organic chemistry, 2) Evaluate the new drug candidates by testing against both susceptible and drug-resistant strains of *Mycobacterium tuberculosis*, and 3) Develop a robust analytical method (near-infrared spectroscopy) to monitor conveniently and rapidly the chemical synthesis of the new compounds. The project is designed to allow feedback from the microbiological testing to guide new synthesis.

Grant: 2R21AI029040-10
Program Director: MILLER, MARISSA A.
Principal Investigator: LEE, JEAN C
Title: GENETIC ANALYSIS OF S. AUREUS CAPSULE PRODUCTION
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 1989/12/01-2001/09/29

Description (Adapted from Applicant's Abstract): *Staphylococcus aureus* is an opportunistic bacterial pathogen responsible for a diverse spectrum of human and animal diseases. Although 11 putative capsular serotypes have been reported, types 5 and 8 constitute about 75 percent of clinical isolates. The type 5 (CP5) and 8 (CP8) polysaccharides are structurally very similar to each other; they differ only in the linkages between the sugars and in the sites of O-acetylation. The overall goal of this study is to use a molecular approach to define the functions, relative biological activity, and regulation of *S. aureus* capsule genes. The molecular events controlling the biosynthesis of CP5 and CP8 will be investigated, and the existence and prevalence of other capsular serotypes will be examined. In the first specific aim, we will determine the enzymatic functions of type 5 capsule genes that are involved in amino sugar biosynthesis: *cap5D*, *cap5E*, *cap5F*, *cap5G*, and *cap5N*. We will express the recombinant proteins in *Escherichia coli* and conduct in vitro assays to evaluate the enzymatic functions of the purified proteins. The second aim will address the biological differences between strains producing type 5 or 8 capsules. We plan to replace the serotype 5-specific capsule genes (*cap5HIJK*) of strain Reynolds with the serotype 8-specific genes (*cap8HIJK*) of strain Becker so that strain Reynolds will produce CP8. The isogenic serotype 5/8 strains will then be compared for quantity of capsule produced, resistance to killing in an in vitro opsonophagocytic killing assay, and virulence in a mouse model of bacteremia and renal abscess formation. The influence of capsule production on the deposition of complement on *S. aureus* cells will also be explored. The third specific aim will examine strains of *S. aureus* that do not react with antibodies to capsule type 1, 2, 5, or 8. Since most of these "non-typeable" strains carry the genes for CP5 or CP8 production, we will determine why capsule is not expressed. We also plan to evaluate non-typeable isolates for the production of capsules other than serotypes 1, 2, 5, and 8. The proposed studies will lead to a better understanding of the organization, structure, function and regulation of capsule expression in *S. aureus*.

Grant: 2R21AI030138-11
Program Director: MILLER, MARISSA A.
Principal Investigator: NOVICK, RICHARD P MD
MICROBIOLOGY:MICROBL
PHYSIOLOGY
Title: MOLECULAR GENETICS OF EXOTOXIN REGULATION IN S. AUREUS
Institution: NEW YORK UNIVERSITY SCHOOL OF NEW YORK, NY
MEDICINE
Project Period: 1991/09/01-2001/09/29

DESCRIPTION (Adapted from the Applicant's Abstract): The overall goal of the project is to understand the regulatory network by means of which staphylococci and streptococci regulate the synthesis of proteins, including virulence factors, that enable the organisms to maintain the state of colonization or to thrive in the hostile in vivo environment during infection. Specific aims are: 1) To determine the mechanism of target gene activation by agr-RNA.; 2) To identify the effectors of virulence gene regulation (a) that respond to inhibitory environmental signals, (b) that are responsible for activation of certain exoprotein genes during early exponential phase and (c) that are responsible for activation of certain exoprotein genes in response to subinhibitory beta-lactam antibiotics; 3) To characterize environmentally determined patterns of virulence gene expression; 4) To identify the genetic regulators and effectors responsible for a) colonization. b) biofilm formation and maintenance. c) intragenetic bacterial interference in staphylococci; 5) To identify and analyze signaling pathways that regulate the production of streptococcal superantigens and possibly other virulence factors. Subcloning, mutation and deletion analysis coupled with the determination of RNA structure will be used to localize functional domains of the agr-determined RNA that regulates virulence gene expression. New regulatory genes will be identified by transposon mutagenesis plus genome-based cloning. Patterns of gene expression under various conditions and in mutant strains will be determined by means of oligonucleotide arrays based on the S. aureus genome. Known regulatory genes will be tested for their effects on colonization, biofilm formation and interference, and new regulatory genes affecting these phenomena will be identified by transposon mutagenesis. The possibility that streptococcal superantigen genes are autoinduced by peptides similar to those of S. aureus will be investigated by the analysis of culture supernatants. These studies have major implications for the control and treatment of infections due to staphylococci and streptococci and possibly other pathogenic bacteria. The most direct of these implications will involve the possibility of blocking the expression of virulence factors by interfering with the signaling pathways by which they are regulated.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2R21AI031431-09
Program Director: BAKER, PHILLIP J.
Principal Investigator: WALKER, DAVID H
Title: EHRLICHIA CHAFFEENSIS SURFACE PROTEINS
Institution: UNIVERSITY OF TEXAS MEDICAL BR GALVESTON, TX
GALVESTON
Project Period: 1991/09/30-2002/04/30

DESCRIPTION (Adapted from the Applicant's Abstract): The long-term goal of this research project is the elucidation of the mechanisms of protective immunity against Ehrlichia chaffeensis, the causative agent of human monocytotropic ehrlichiosis (HME). Achievement of this goal requires knowledge of the ehrlichial antigens that stimulate protective immunity and of the humoral and cellular immune mechanisms that are effective in the clearance of ehrlichiae from the infected macrophages throughout the body. HME is a life-threatening tick-borne infection that has been associated with toxic shock syndrome-like manifestations, adult respiratory distress syndrome, meningitis, and disseminated intravascular coagulation in immunocompetent patients, overwhelming opportunistic infection in immunocompromised patients, and a fatality rate of 2.7%. The specific aims, designed to test the hypothesis that the immunodominant, surface-exposed p 120 and p28-family stimulate protective immunity by a combination of antibodies and cellular mechanisms, are 1) to determine the roles of proteins of the p28 family and p120 in stimulating protective immunity in a mouse model of HME against an organism closely related to E. chaffeensis, an Ehrlichia species isolated from Japanese Ixodes ovatus ticks (IOE); 2) to determine the importance and mechanism(s) of antibodies in protective immunity against IOE in the mouse model of HME; and 3) to identify the cellular immune mechanisms that are critical in protective immunity against IOE in the mouse model of HME. The research design includes purification of recombinant p120 and each member of the p28 family of IOE, production of DNA vaccines expressing each of these proteins, and testing the DNA and recombinant protein vaccines in the highly pathogenic IOE C57BL/6 mouse model. The hypothesis of humorally mediated immunity will be examined by passive immunization studies with specific polyclonal and monoclonal antibodies to p 120 and the p28 family in IOE-challenged immunocompetent, SCID, and Fc-receptor knockout mice. Opsonization will be investigated in murine and human macrophages in vitro with E. chaffeensis and specific polyclonal and monoclonal antibodies. Cellular immune mechanisms will be elucidated using gene knockout mice (MHC Class I, MHC Class II, 8 T-cell receptor, IFN- γ , perforin, INOS), immunodeficient SCID and Beige, and TNF- α depleted mice, immunohistochemical and flow cytometric analyses of the cell subsets and their cytokine profiles, adoptive transfer of T-lymphocytes and their subsets including antigen-specific T-cell lines, and determination of the chemokines that are important for immunity to the IOE in an outstanding new mouse model of HME.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2R21AI033516-07
Program Director: MILLER, MARISSA A.
Principal Investigator: MURRAY, BARBARA E MD INTERNAL
MED:INFECTIOUS DISEASE
Title: GENETIC ANALYSIS OF ENTEROCOCCI
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX
HOUSTON
Project Period: 1993/09/30-2001/02/14

DESCRIPTION (Applicant's Abstract): Enterococci are the third most common cause of endocarditis, behind streptococci and staphylococci, and the second or third most common cause of hospital acquired infections; among these, *Enterococcus faecalis* is the predominant species isolated. Antimicrobial resistance likely enhances the role of enterococci in nosocomial infections and certainly make it more difficult to successfully treat patients, particularly those with endocarditis. The central hypothesis of this work is that by better understanding enterococci, new therapeutic or preventative modalities can be developed. Work during the current funding period has identified and characterized a number of antigen encoding genes; a polysaccharide gene cluster (*epa*) that appears to influence virulence in mice; different adherence phenotypes and gene, *ace*, that appears to be involved in adherence; and a gene locus with homology to the accessory gene regulator (*agr*) locus of staphylococci that appears to regulate an enterococcal gelatinase and a serine protease that also influence virulence in mice. In the renewal application, the investigators propose (1) to verify that the *agr*-like locus regulates *gelE* and *sprE* and if all are important for virulence; to determine the commonality of these genes among *E. faecalis*, and to determine how the enterococcal *agr*-like locus is regulated and if it, like staphylococcal *agr*, regulates other genes. They also plan (2) to test the hypothesis that *Ace* (a newly described adhesin for collagen of enterococci) is the cause of the adherence they have reported and is important for virulence; to explore the regulation of *Ace* production; and to determine the distribution and effect of variations in *ace*, if *Ace* elicits an antibody response in humans (using recombinant *Ace* and patient sera, including over 17 from patients with *E. faecalis* endocarditis) and if antibody made during infection, or antibody to recombinant *Ace*, is protective. In their third specific aim, they plan (3) to establish if the polysaccharide gene cluster is the cause of a recently described mucoid phenotype, to study its regulation, and to further test its contribution to adherence to foreign material, virulence and protection. In their "Methodologic" Specific Aims, they plan (4) to devise a system for constructing non-polar deletion mutants using counter-selection, based on their prior work with *E. faecalis* *pyr* mutants, and to develop additional assays to look at bacterial persistence and less acute infections that would help us avoid lethality models. They hope that results from this work will provide solid leads in their quest for methods to prevent, control, or combat *E. faecalis* infections.

Grant: 2R21AI035237-07
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: GROS, PHILIPPE PHD
Title: NRAMPL AND PHAGOCYTE FUNCTION
Institution: MC GILL UNIVERSITY MONTREAL, PQ
Project Period: 1993/09/30-2001/06/30

DESCRIPTION (Adapted from the Applicant's Abstract): Infectious diseases have re-emerged as a major health problem in North America, in part due to the widespread emergence of antibiotic resistance. Tuberculosis is a dramatic example of this threat, with the appearance of highly virulent and multidrug resistant *M. tuberculosis*, and the prevalence of TB in AIDS patients. The mechanisms of defense against intracellular parasites, and the bacterial strategies underlying survival and replication in host phagocytes remain poorly understood. A better understanding of host defenses against such infections may suggest new strategies for intervention in these diseases. Using a genetic approach, the PI has identified a new component (Nramp1) of anti-microbial defenses of phagocytes. Mutations at Nramp1 in mice cause susceptibility to several intracellular infections, and polymorphic variants at human NRAMP1 are associated with susceptibility to TB and leprosy in endemic areas of disease. Nramp1 is part of a large family of membrane transporters that has been highly conserved from bacteria to man. Nramp1 is expressed in the lysosomal compartment of macrophages and is targeted to the membrane of bacterial phagosomes soon after phagocytosis. By homology with the known substrates of other Nramp family members, it is proposed that Nramp1 functions as a divalent cation efflux pump at the phagosomal membrane to suppress bacterial replication. The current proposal has five major goals. The first is to study in the mouse the role of Nramp1 and other genes in regulating *Mtb* replication in the lungs. The second is to understand how Nramp1 delivery affects the physiological properties of the phagosome including maturation, acidification and bactericidal activity. The role of Nramp1 in neutrophil function will also be studied. The third goal is to identify the substrate and mechanism of transport of Nramp1 at the phagosomal membrane. The fourth is to identify protein determinants responsible for Nramp1 targeting to the lysosome and residues essential for substrate binding and transport. The fifth is to study the role of the close Nramp2 homologue in divalent cation transport in normal tissues, including at the phagosomal membrane. Together, these studies should clarify the role and mechanism of action of Nramp1 in phagocyte anti-microbial defenses, which may in turn suggest new avenues for intervention in TB and other infectious diseases.

Grant: 2R21AI037601-07
Program Director: BAKER, PHILLIP J.
Principal Investigator: LEONG, JOHN M MD
Title: HOST CELL INTERACTIONS BY PATHOGENIC BORRELIAE
Institution: UNIV OF MASSACHUSETTS MED SCH WORCESTER, MA
WORCESTER
Project Period: 1995/04/01-2001/03/31

Borrelia burgdorferi is the causative agent of Lyme disease, and *B. hermsii* and *B. turicatae* are causative agents of tick-borne relapsing fever. Pathogen-host cell interactions are thought to be critical determinants of the site and severity of infection, and we have focused on *Borrelia* recognition of two classes of host cell molecules: (1) glycosaminoglycans (GAGs); and (2) integrins and their associated proteins. For *B. burgdorferi*, we found that differences in GAG recognition were associated with differences in host cell type-specific binding, and identified a surface protein, Bgp, that may be the major *B. burgdorferi* GAG receptor. This bacterium also recognizes the activation-dependent platelet integrin $\alpha\text{IIb}\beta\text{3}$, and thereby selectively binds to activated (vs. resting) platelets. This integrin-binding activity is predicted to target the Lyme disease spirochete to the vessel wall at sites of platelet adherence, and could explain a salient feature of Lyme disease: vascular pathology of the arterial circulation. In our studies of relapsing fever spirochetes, high-level GAG-binding correlated with high-level growth in the bloodstream and a variable major protein, VspB, promoted attachment to GAGs. Additionally, to contrast to *B. burgdorferi*, *B. hermsii* bound and activated resting platelets. This contact-dependent platelet activation activity was apparently mediated by the integrin-associated platelet signaling molecule CD9. Relapsing fever spirochetes have also been shown to bind to erythrocytes, and we speculate that attachment of relapsing fever spirochetes to circulating blood cells such as platelets and erythrocytes could promote the retention and high level growth of the bacteria in the blood. Interaction of spirochetes with these two cell types could also contribute to two common manifestations of relapsing fever: anemia and thrombocytopenia. Thus, we have formulated working models for the role of host cell binding in colonization and disease expression by pathogenic *Borrelia*. To test predictions of these models, the following questions will be addressed: (1) does reducing the spirochete-platelet interactions affect colonization of the vessel wall and heart in Lyme disease or thrombocytopenia in relapsing fever? (2) does CD9 play a role in platelet activation by *B. hermsii*? (3) does the GAG-binding activity of the relapsing fever spirochete influence cell attachment and growth in the bloodstream of infected animals? (4) does Bgp play a predominant role in GAG binding by *B. burgdorferi*? The proposed experiments may uncover both potential therapeutic strategies for combating *Borrelia* infections as well as general principles that govern tissue-specific infection by bacterial pathogens.

Grant: 2R21AI037744-06
Program Director: BAKER, PHILLIP J.
Principal Investigator: SAMUEL, JAMES E PHD
Title: PATHOGENIC ROLES OF COXIELLA BURNETTI SURFACE PROTEINS
Institution: TEXAS A&M UNIVERSITY HEALTH SCIENCE COLLEGE STATION, TX
CTR
Project Period: 1995/07/15-2002/09/26

DESCRIPTION (Adapted from the Applicant's Abstract): *Coxiella burnetii*, the etiologic agent of Q fever, is an obligate intracellular bacterium 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. The author proposes that survival mechanisms are the principle virulence determinants of *C. burnetii*. Four strategies will be evaluated for their contribution to survival. First, the applicant plans to define the nature and role of the *C. burnetii* lifecycle in survival. 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 in the 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, he 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. The author has begun to define proteins involved in iron acquisition using a ferric uptake regulator titration assay (FURTA). Third, he plans to characterize the role of anti-oxidant gene products in survival. The working hypothesis is that *C. burnetii* express enzymes that 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 is expected to pave the way for characterizing catalase, periplasmic superoxide dismutase (Cu/Zn SOD) and RecA. He will test molecular Koch's postulate for the requirement of RecA by creating a transdominant negative mutant through genetic transformation. Finally, he will characterize the role that acid phosphatase plays in intracellular survival. The working hypothesis is that *C. burnetii* expresses acid phosphatase which phosphorylates phagolysosomal proteins and reduces their antibacterial activity. The author predicts that his approach will define major survival and virulence determinants. The results are expected to be significant, as *C. burnetii* is a unique and valuable comparative model for intracellular parasitism. In addition, *C. burnetii* is a common although infrequently diagnosed agent that causes fever of unknown origin and a potential bioterrorist agent. Identification of primary virulence determinants may provide focus for therapeutic, diagnostic and vaccine development.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R21AI044868-01A2
Program Director: RUBIN, FRAN A.
Principal Investigator: FOXMAN, BETSY
Title: MOLECULAR EPIDEMIOLOGY OF GROUP B STREPTOCOCCUS
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2000/09/29-2002/09/28

Group B streptococcus (GBS) is a major cause of neonatal sepsis and meningitis, and causes a number of potentially life-threatening illnesses among adults. GBS colonization among pregnant women increases risk of GBS colonization and subsequent disease among the infant. A better understanding of both behavioral and bacterial risk factors for acquisition and long-term colonization is essential to appropriately plan and target intervention strategies, including predicting the impact of vaccination on the circulation of various serotypes in the population. Further, a description of the prevalence of GBS serotypes in the population is required to formulate a policy for the use of GBS vaccine. Our overall goal is to describe the prevalence and incidence of GBS among 500 male and 500 female college students, and to identify behavioral and bacterial factors associated with GBS vaginal, urethral and bowel colonization, acquisition and loss, and describe the prevalence and incidence of colonization by pulsed-field gel electrophoresis (PFGE) pattern, serotype, and presence of GBS virulence factors. All participants will be screened for GBS at enrollment, and 2 and 4 months post enrollment. We will invite participants acquiring GBS during the study to recruit their most recent sex partner for study and to return for an additional visit at 6 months following enrollment. Specifically we plan to: Aim 1: Estimate the prevalence of vaginal, urethral and rectal colonization with GBS by serotype and PFGE pattern among adult males and adult, non-pregnant females. Aim 2: Estimate the incidence of GBS acquisition and loss by GBS serotype, PFGE pattern and site of colonization among males and females over a 6 month period. Aim 3: Estimate the duration of GBS colonization by serotype, PFGE pattern and site of colonization among males and females over a 6 month period. Aim 4: Using PFGE, determine whether sex partners of participants who acquire GBS during the study period are colonized with the same GBS strain as the study participant. In addition, we will describe the association of behavioral and socio- demographic characteristics, and medical and social history with prevalence, incidence and duration of colonization with specific GBS strains as defined by serotype and PFGE pattern.

Grant: 1R21AI045732-01A1
Program Director: KLEIN, DAVID L
Principal Investigator: CARBONETTI, NICHOLAS H PHD
Title: PERTUSSIS TOXIN TRANSPORT IN CELLS AND EPITOPE DELIVERY
Institution: UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD
SCHOOL
Project Period: 2000/09/15-2002/09/14

Description (Adapted from the applicant's abstract): The long term goal of this project is to develop vaccine molecular using a genetically detoxified bacterial exotoxin, pertussis toxin (PT*), as a vector molecule for induction of antigen-specific CD8+ T cell responses, including cytolytic T lymphocytes (CTL), which can protect against viral and parasitic infections. CTL recognize antigens as peptides in association with major histocompatibility complex (MHC) class I molecules on the surface of infected cells. The candidate vaccine molecules will consist of genetically constructed fusions of peptide or polypeptide antigens with PT*, to stimulate antigen-specific CD8+ CTL responses in vivo. PT, like several other bacterial toxins, enters cells as part of its intoxication mechanism, and therefore can be exploited as an intracellular delivery molecule for antigens. We have found that PT* can deliver epitope peptides to MHC class I molecules in target cells for presentation to CTL, but that this apparently does not involve the endogenous cytosolic pathway of MHC class I antigen processing and presentation, to the endoplasmic reticulum (ER). We hypothesize that PT* can deliver epitomes to class I because of its retrograde transport in cells to the ER. We therefore aim to elucidate the intracellular transport pathway utilized by PT* to determine the mechanism by which it delivers epitopes to class I. This information will allow us to enhance this process, and therefore also to improve CTL responses, by modifying PT* to alter its intracellular transport. Such modified PT* molecules could represent powerful CTL-stimulating vaccine vector molecules. The fusion molecules will be tested in model systems in vitro and in vivo to assess their class I targeting capacity, their immunogenicity in vivo and their protective capacity in an animal model. The specific aims of this application are as follows. (1) To elucidate the intracellular transport pathway utilized by PT*. (2) To test and improve the capacity of PT*-epitope fusion molecules to stimulate epitope-specific CTL responses in vitro. (3) To test the capacity of PT*-epitope fusion molecules to stimulate epitope-specific CTL responses in mice and to protect mice from virus challenge. The advantages of PT* as a vaccine vector include its potent adjuvant-icity and its safety and non-reactogenicity for humans, being a component of a licensed pertussis vaccine.

Grant: 1R21AI045836-01A1
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: CARLIN, JOSEPH M PHD
Title: CHLAMYDIAL EVASION OF IFN-MEDIATED IMMUNITY
Institution: MIAMI UNIVERSITY OXFORD OXFORD, OH
Project Period: 2000/06/01-2001/05/31

This proposal is in response to the program announcement "Research on molecular immunology of STDs (ROMIS)". Interferon (IFN)-gamma induces an effective antichlamydial mechanism in vitro by inducing indoleamine 2,3-dioxygenase (IDO) which depletes tryptophan that is essential for chlamydial growth. Although proinflammatory cytokines produced during infection enhance the amount of IDO induced by IFN, the presence of chronic disease suggests that Chlamydia is evading this response. The goals of this research project are to identify and characterize mechanisms by which Chlamydia evades the effect of IFN. Chlamydia may be affecting IDO regulation directly by interfering with transcriptional activation of the IDO gene by IFNs, or by blocking the effect of proinflammatory cytokines. Chlamydia also may be regulating IDO indirectly by stimulating production of interleukin-10 (IL-10) leading to inhibition of IDO transcription. Specific aim 1: Molecular mechanisms of IDO potentiation. IDO regulatory mechanisms will be evaluated using HeLa cells transfected with a green fluorescent protein reporter vector containing the IDO promoter. Identification of IDO promoter regions and DNA-binding proteins will be by EMSA and super-shift assays. Site-directed mutagenesis will be used to confirm promoter site function. Specific aim 2: Direct mechanisms of evasion. The effect of Chlamydia on IDO promoter activity and cytokine receptor expression will be assessed using two-color flow cytometric analysis of infected HeLa cells. Specific aim 3: Indirect mechanisms of evasion. The role of IL-10 in inhibition of IDO will be assessed by quantifying IL-10 production by Chlamydia-exposed cells using ELISA, assessing the effect of IL-10 on proinflammatory cytokine production by Chlamydia-exposed cells and by measuring the effect of IL-10 on IDO regulation using the fluorescent IDO promoter reporter. Thus, the aims are dissect the process of IDO potentiation at the transcriptional level, and to assess the means by which Chlamydia interferes with this process. Accomplishment of the aims will help resolve the long-term objectives of this research project: to determine how Chlamydia evades and otherwise effective immunologic response, and to understand regulation of this response in order to overcome Chlamydia's evasive mechanisms.

Grant: 1R21AI046543-01A1
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: HAUSER, ALAN R MD
Title: PSEUDOMONAS AERUGINOSA TYPE III SECRETION AND APOPTOSIS
Institution: NORTHWESTERN UNIVERSITY CHICAGO, IL
Project Period: 2000/09/29-2002/09/28

DESCRIPTION (Adapted from the Applicant's Abstract): *Pseudomonas aeruginosa* is one of the more important nosocomial pathogens, accounting for 10% of all hospital-acquired infections. The frequency and severity of *P. aeruginosa* infections underscore the need to understand better the pathogenic mechanisms utilized by this pathogen. Recently, a type III secretion system has been discovered in this organism and shown to play a role in disease. Characterization of the system has already identified several interesting secreted toxins, and further examination is likely to find more. The hypothesis to be evaluated is that the *P. aeruginosa* type III system, like the type III system of several other gram-negative bacteria, secretes a factor that causes apoptosis of eukaryotic cells and plays a role in virulence. The PI proposes the following specific aims to evaluate this hypothesis: 1) Genetic and biochemical approaches will be utilized to identify the putative apoptosis-causing factors, which shall be referred to as ExoZ. 2) ExoZ will be introduced into eukaryotic cells to determine if it by itself is sufficient to cause apoptosis. 3) The role of ExoZ in disease will be investigated by comparing an isogenic mutant with the wild type parental strain in a mouse model of acute pneumonia. If initial attempts to identify ExoZ are not successful, the approaches used in specific aim 1 will be broadened to identify other cytotoxic factors, one of which will be chosen for in-depth analysis in specific aim 2 and 3. Successful completion of these specific aims will result in the identification and preliminary characterization of a *P. aeruginosa* type III secreted factor that causes apoptosis or is otherwise cytotoxic and the determination of this factor's role in disease. Such information will aid in the better understanding of *P. aeruginosa* pathogenesis and lead to novel therapeutic interventions. In addition, new insights gained from this work will lay the foundation for future investigations regarding the interaction of this factor with eukaryotic apoptosis cascades and the role of apoptosis in bacterial infections. Such advances are urgently needed given the increasing frequency of antibiotic resistance in *P. aeruginosa* clinical isolates.

Grant: 1R21AI046706-01
Program Director: MILLER, MARISSA A.
Principal Investigator: PIER, GERALD B PHD MICROBIOLOGY, OTS
Title: VIRULENCE AND IMMUNITY TO STAPHYLOCOCCI
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 2000/01/10-2000/12/31

The long-term goal of this study is to understand the role in pathogenesis and immunity of an environmentally-regulated, in vivo expressed surface polysaccharide of *Staphylococcus aureus* chemically characterized as poly-N-succinyl-beta-1-6 glucosamine (PNSG). PNSG has previously been determined to be the protective capsular polysaccharide/adhesin (PS/A) antigen of *Staphylococcus epidermidis*, raising the possibility that PNSG could be used as a "pan-staphylococcal" vaccine. To define the role of PNSG in pathogenesis of *S. aureus* infection 5 different PNSG-deficient *S. aureus* strains will be constructed by genetic means via interruption of the genes in the intracellular adhesin (*ica*) locus that encodes proteins needed for synthesis of PNSG. Isogenic parental, mutant and *ica* complemented strains will be evaluated in vitro to determine the role of PNSG in promoting *S. aureus* adherence to catheters and in providing resistance of bacterial cells to phagocytic killing by leukocytes and complement. The same strains will also be tested for infectious capability in 5 different of animal models of *S. aureus* infection. The models encompass nasal colonization, acute lethality, kidney abscess formation, endocarditis and localized skin abscesses. Because PNSG isolated from some strains of staphylococci have up to 30 percent of the succinate substituents on the polyglucosamine backbone replaced by acetate, purified PNSG, with differing ratios of succinate and acetate substituents on the polyglucosamine backbone, will be produced for immunologic studies in experimental animals. Rabbits will be immunized with the variants and sera assessed for antibody titer and opsonic killing ability. The PNSG variant structures will also be used to immunize mice to evaluate the ability of these constructs to generate protective immunity in the same models used for the study of the role of PNSG in *S. aureus* virulence. In addition, passive protective capacity of the rabbit sera raised to the variant PNSG constructs will be evaluated in the 5 animal models. All the above mentioned studies will provide new and useful information regarding pathogenesis and immunity of staphylococcal infections, because it has not previously been appreciated that *S. aureus* expresses the PNSG antigen as a surface, capsule-like polysaccharide. By the end of these studies we expect to have a clear understanding of the role of PNSG in virulence, as determined in a variety of staphylococcal infection models, the immunochemical properties of PNSG that can engender protective immunity, and the types of *S. aureus* infections wherein PNSG- specific immunotherapies show the most potential for success.

Grant: 1R21AI049840-01
Program Director: LANG, DENNIS R
Principal Investigator: CHRISTIE, GAIL E PHD OTHER AREAS
Title: PHAGE ENCODED FUNCTIONS IN ENTEROHEMORRHAGIC E.COLI
Institution: VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA
Project Period: 2000/09/30-2002/09/29

DESCRIPTION (adapted from the application) The long-term goal of this research is to understand the role of temperate phages in, and the contribution of phage-encoded gene products to, the virulence of enterohemorrhagic Escherichia coli (EHEC). EHEC are emerging foodborne pathogens that have caused large-scale outbreaks of gastrointestinal illness in developed countries during the past two decades. Intestinal infection can lead to diarrhea, hemorrhagic colitis or more severe systemic complications, such as hemolytic uremic syndrome. The production of Shiga toxins by EHEC strains plays an important role in the development of serious complications following EHEC infection. Two immunologically distinct Shiga toxins, designated Stx1 and Stx2, have been identified among clinical E. coli isolates of many serotypes. Genes for both Shiga toxin types in E. coli have been shown to be encoded on lysogenic lambdoid bacteriophages. These Shiga toxin-encoding phages have played an important role in transmitting the stx genes during the evolution of Stx-producing enteric pathogens, and continue to be involved in ongoing dissemination of Shiga toxin genes to new hosts. In addition, recent studies have shown that the toxin genes appear to be integrated into the lytic circuitry of these phages in such a way that prophage induction leads to increased toxin gene expression and concomitant release of toxin by host cell lysis. One aim of the experiments outlined in this application is to define more clearly the roles of phage-encoded functions in toxin gene expression and toxin release. A second aim of the application is to investigate other phage-encoded genes that are postulated to play roles in lysogenic conversion. The products of these genes may affect processes, such as colonization or immune evasion, that could contribute to the virulence of lysogenic bacteria. Using the Stx2-encoding phage 933W, which has been sequenced in its entirety, directed mutations in individual genes will be constructed. The effects of these mutations on Shiga toxin production or other interactions with host cells will be assessed in vitro, and effects on virulence using a mouse model system will be determined.

Grant: 2R37AI020384-17
Program Director: BAKER, PHILLIP J.
Principal Investigator: WOOD, DAVID O PHD MICROBIOLOGY, OTS
Title: GENETIC ANALYSIS OF RICKETTSIA PROWAZEKII
Institution: UNIVERSITY OF SOUTH ALABAMA MOBILE, AL
Project Period: 1983/07/01-2005/06/30

DESCRIPTION (Adapted from the Applicant's Abstract): Members of the genus *Rickettsia* are the etiologic agents of rocky mountain and other spotted fevers and endemic, scrub and epidemic typhus, diseases that pose a pernicious health threat worldwide. *Rickettsia prowazekii*, the etiologic agent of epidemic typhus is an obligate intracellular parasitic bacterium that can grow only within the cytoplasm of a eucaryotic host cell. The ability of rickettsiae to exploit this intracellular niche in animals as diverse as arthropods and humans and to subsequently cause serious human disease provides the impetus for this study. This proposal focuses on the development and application of genetic techniques to address questions regarding the pathogenic bacterium *R. prowazekii* and its obligate intracytoplasmic existence. It exploits the availability of the *R. prowazekii* genome sequence and the development of rickettsial genetic technologies to test hypotheses related to rickettsial gene function, DNA replication, and pathogenic mechanisms. In Specific Aim 1 the PI's goal is to capitalize on a rickettsial transformation system and identification of a selectable antibiotic resistance gene that can be expressed in *R. prowazekii* to discriminate, via knockouts, essential function at the level of single genes. Specifically targeted genes include those that encode products with homology to known virulence genes of other bacteria, genes hypothesized to be expressed only in the arthropod vector, genes hypothesized to be non-functional and part of the process of rickettsial reductive evolution, and finally, genes with homologs within the *R. prowazekii* genome. In addition, a transposon-based approach will be used to generate random insertion mutants. In Specific Aim 2, the PI's goal is to isolate the functional origin of replication. One approach will attempt to generate a rickettsial mini-chromosome by linking putative origin fragments with the selectable erythromycin-resistant gene, *ereB*. An alternate method will identify the origin by binding of rickettsial DnaA. Specific Aim 3 will continue the PI's characterization of transcription termination and identification of rickettsial transcriptional changes that occur just prior to lysis of the host cell. Using ribonuclease protection studies, the PI will determine whether these changes reflect a general property of the rickettsiae by examining additional non-intrinsic termination sites and the effect of cell number on termination at these sites. Modulation of Rho and its correlation to these changes will be addressed.

Grant: 2R37AI022931-15
Program Director: MILLER, MARISSA A.
Principal Investigator: SELSTED, MICHAEL E MD
Title: MOLECULAR ASPECTS OF LEUKOCYTE ANTIMICROBIAL PEPTIDES
Institution: UNIVERSITY OF CALIFORNIA IRVINE IRVINE, CA
Project Period: 1989/07/01-2005/03/31

Abstract Text Not Available

Grant: 2R37AI023538-14
Program Director: LANG, DENNIS R
Principal Investigator: ISBERG, RALPH R BA
Title: MOLECULAR BASIS OF YERSINIA/HOST CELL INTERACTION
Institution: TUFTS UNIVERSITY BOSTON BOSTON, MA
Project Period: 1986/06/01-2005/05/31

DESCRIPTION (Adapted from the Applicant's Abstract): The long-term objectives of these studies are to determine how an enteropathogenic bacterium is able to enter within host cells and to determine the cellular route taken by such pathogens after oral inoculation. *Yersinia pseudotuberculosis* is being studied in order to gain detailed information on the function of bacterial- and host-encoded internalization factors. To this end, the binding of the bacterial invasin protein with its mammalian integrin receptors, and the consequences of this event within the host cell, will be studied. In addition, this proposal will analyze the nature of the events that occur shortly after the bacterium is ingested by the host, when invasin appears to exert its most important role in disease. Efficient uptake promoted by invasin is regulated by the host cell FAK and Rac1 proteins, and requires high affinity binding to integrin receptors as well as multimerization of the invasin cell adhesion domain. To probe the molecular mechanism of uptake promoted by the substrate-receptor pair, and analyze the events that occur during translocation of the organism across the intestinal epithelium, the following studies will be performed: 1) using the recently completed crystal structure, determinants within invasin that allow uptake and differentiate invasin from other integrin substrates will be identified; 2) the region of invasin that allows presentation of the cell binding motif will be identified and analyzed topologically using genetic strategies; 3) the role of FAK in promoting uptake will be determined by analyzing its role in regulating both the nature of the phagocytic cup and the mobility of integrins within the membrane; 4) the route that *Y. pseudotuberculosis* takes to cross the intestinal epithelium in the absence of invasin expression will be determined. It is now apparent that many bacterial pathogens initiate disease by entering within host cells, and after ingestion, enteropathogens are able to translocate across multiple sites in the intestine. Therefore, these studies will result in determining how an enteric disease by a bacterial pathogen is initiated, and provide insight into a step that could be blocked by chemotherapeutic agents prior to colonization by such microorganisms. In addition, identification of the components that allow a simple organism to enter an animal cell could result in new techniques to introduce therapeutic agents that would otherwise not be able to enter the host cell.

Grant: 2R37AI030492-12
Program Director: LANG, DENNIS R
Principal Investigator: GALAN, JORGE E DVM VETERINARY
MEDICINE
Title: MOLECULAR GENETIC ANALYSIS OF SALMONELLA CELL INVASION
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 1991/01/01-2005/06/30

Salmonella continues to be a major world-wide health concern. Essential to the pathogenicity of *Salmonella enterica* is the function of a type III secretion system encoded within a pathogenicity island (SPI-1) of its chromosome. This system mediates the delivery into the host cell of bacterial effector proteins which stimulate host cell responses including actin cytoskeleton rearrangements leading to bacterial uptake, production of pro-inflammatory cytokines, and the characterization of the structural components of this secretion system, the identification of associated proteins that aid the secretion process, and the identification of secreted proteins that stimulate or interfere with cellular responses. We have also established that some of the components of the type III secretion apparatus are organized in a supramolecular structure, the needle complex, that spans the bacterial envelope and resembles the flagellar basal body. The proposed research project, a natural extension of the previous studies, is aimed at gaining a better understanding of the function of the centisome 63 type III secretion system of *Salmonella enterica*. More specifically we propose: 1) To study the composition of the type III secretion-associated needle complex; 2) To investigate the function of InvC, the type III secreted-associated ATPase that is presumed to energize the secretion machinery; 3) To identify the signals that allow the recognition of different secreted proteins by the secretion apparatus; and 4) To investigate the regulatory function of SicA, a type III secretion-associated chaperone. These studies will enhance our understanding of the interaction of *Salmonella* with host cells. Since type III secretion systems are present in several important pathogenic bacteria, this studies may also help the development of novel antimicrobial drugs potentially effective against many bacterial pathogens.

Grant: 1R37AI047923-01
Program Director: MILLER, MARISSA A.
Principal Investigator: MURRAY, BARBARA E MD INTERNAL
MED:INFECTIOUS DISEASE
Title: ALTERNATIVE APPROACHES FOR E FAECALIS INFECTIONS
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX
HOUSTON
Project Period: 2000/08/01-2005/07/31

DESCRIPTION (Adapted from the Applicant's Abstract): Enterococci are the 3rd most common cause of endocarditis, behind streptococci and staphylococci, and the 2nd or 3rd most common cause of hospital acquired infections, with *Enterococcus faecalis* being the predominant species isolated. Antimicrobial resistance likely facilitates the establishment of enterococci in nosocomial infections and certainly makes it more difficult to successfully treat patients, particularly those with endocarditis. The central hypothesis of this project is that by better understanding enterococci, new therapeutic or preventative modalities can be developed. Work during a previously funded grant identified and characterized a number of antigen encoding genes; a polysaccharide gene cluster (*epa*) that appears to influence virulence in mice; different adherence phenotypes, and a gene, *ace*, that appears to be involved in adherence; and a gene locus with homology to the accessory gene regulator (*agr*) locus of staphylococci that is involved in expression of an *E. faecalis* gelatinase and a serine protease that also influence virulence in mice. In this application, the investigators propose (1) to verify that the *E. faecalis* *agr*-like locus regulates *gelE* and *sprE* and determine if all are important for virulence; to investigate the distribution of these genes among *E. faecalis*; and to determine how the enterococcal *agr*-like locus is regulated and if it, like the staphylococcal *agr*, regulates other genes. They also plan (2) to test the hypothesis that *Ace* (a newly described adhesin for collagen of enterococci) is the cause of the adherence we have reported and is important for virulence; to explore the regulation of *Ace* production; and to determine the distribution and effect of variations in *ace*, if *Ace* elicits an antibody response in humans (using recombinant *Ace* and patient sera) infected by *E. faecalis* and if antibody made during infection, or antibody to recombinant *Ace*, is protective. In their third specific aim, they plan (3) to establish if the polysaccharide gene cluster is the cause of a recently described mucoid phenotype, to study its regulation, and to further test its contribution to adherence to foreign material, virulence and protection. They will also explore a system for constructing non-polar deletion mutants using counter-selection based on their prior work with the *E. faecalis* *pyr* genes, and to explore additional assays that would help to avoid lethality models. The investigators hope that results from this work will provide solid leads in the quest for methods to prevent, control, or combat *E. faecalis* infections.

Grant: 1U01AI045667-01A1
Program Director: MILLER, MARISSA A.
Principal Investigator: GILL, STEVEN R PHD
Title: GENOME ANALYSIS OF STAPHYLOCOCCUS EPIDERMIDIS
Institution: INSTITUTE FOR GENOMIC RESEARCH ROCKVILLE, MD
Project Period: 2000/04/01-2002/03/31

The goal of this project is to determine the complete genome sequence of *Staphylococcus epidermidis*, a coagulase negative staphylococci (CoNS) which is an emerging pathogen of increasing clinical significance. Of the CoNS group, *S. epidermidis* is the most virulent and the most common isolate from human infections. The infections caused by *S. epidermidis* include bacteremia, osteomyelitis, pyoarthritis and foreign body infections. Emergence of *S. epidermidis* as a significant pathogen has been accompanied by a concurrent increase in methicillin resistant isolates (MRSE) and clinical isolates with intermediate resistance to vancomycin. Because *S. epidermidis* often serves as a reservoir for resistance determinants that are shared with *Staphylococcus aureus*, the emergence of strains fully resistant to vancomycin undoubtedly represents one of the most frightening challenges in antibiotic resistance. Clearly, the complete genome sequence and a set of recombinant clones would provide a tremendous resource for the study of *S. epidermidis*. The *S. epidermidis* strain chosen for this project is RP62A, a well characterized, methicillin resistant, slime producing strain that is pathogenic to humans and virulent in animal infection models. The approach will be a modified whole genome random sequencing strategy successfully used at TIGR to completely sequence eight prokaryotic genomes. The project will consist of four phases: 1) construction of random small and medium insert plasmid libraries and a large insert BAC library from *S. epidermidis* RP62A, 2) sequencing both ends of approximately 20,250 small insert clones (40,500 sequence fragments), 3) sequencing the ends of a set of minimally overlapping BAC clones and medium size plasmid clones to provide a scaffolding structure that will minimize the effort required for gap closure and provide confirmation of the underlying assembled structure, and 4) assembly and annotation of the genome to identify structural features, assign gene and functional roles to open reading frames based on database similarity searches. This project will complement TIGR's ongoing *S. aureus* sequencing project and enable us to determine virulence factors unique to each species. This project will enable us to identify the virulence factors, their regulatory networks and pathogenic mechanisms used by *S. epidermidis* to invade and infect human tissue. The data developed from this study will be deposited into a variety of databases including the TIGR World Wide Web site. In addition, small insert plasmid and BAC clone sets will be made available to the research community.

Grant: 1U01AI046517-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: FLEISCHMANN, ROBERT D PHD
Title: THE COMPLETE GENOME SEQUENCE AND ANALYSIS OF M SMEGMATIS
Institution: INSTITUTE FOR GENOMIC RESEARCH ROCKVILLE, MD
Project Period: 2000/04/01-2003/03/31

The completion and annotation of the DNA sequence from two strains of *M. tuberculosis*, the laboratory strain H37Rv and the virulent clinical isolate CDC1551 will provide the mycobacteria and tuberculosis research communities with an unprecedented resource for investigating the infectivity, virulence, colonization, pathogenicity, and the mechanisms of drug- sensitivity and resistance of *M. tuberculosis*. Despite the abundance of information provided by the genomic sequence, the exceedingly slow growth rate and highly virulent nature of the organism make working with *M. tuberculosis* and its genetic manipulation formidable tasks. A great deal of effort has gone into developing *M. smegmatis*, a fast-growing non-pathogen, as a genetic surrogate for *M. tuberculosis*. Through these efforts, it is possible for the mycobacterial researcher to utilize *M. smegmatis* in much the same manner as *E. coli* is used in the greater microbiological community. Obtaining the complete genome sequence of *M. smegmatis* will represent a milestone in mycobacterial research. In addition to providing invaluable information for continuing its development as a genetic system for recombinant methodologies, the organism provides a valuable model for studying unique aspects of mycobacterial biology, including cell wall biosynthesis, regulation of growth-rate, drug-sensitivity, protein secretion, gene regulation, etc. The Institute for Genomic Research has developed a cost effective and efficient approach to microbial genome sequencing that has been used to sequence and assemble seven microbial genomes. The genome of *M. smegmatis* strain mc2155 will be sequenced using this whole genome shotgun strategy. Small and large insert random libraries will be prepared and a sufficient number of random shotgun sequences to reach 8-fold coverage will be obtained. Assembly of the genome will be done using the version 2.0 of the TIGR Assembly software and closure will be obtained with a variety of PCR based techniques developed at The Institute. Annotation of the genome will employ a variety of computer techniques for identifying open reading frames and identifying proteins of known function through similarity searches against curated databases. Additional analyses will identify features such as exported proteins, tRNA and rRNA genes, repeated sequences, promoter sequences and ribosome binding sites. The data will be made available to the research community through the TIGR Microbial Database on the World Wide Web (www.tigr.org).

Grant: 1U01AI047409-01
Program Director: GOTTLIEB, MICHAEL
Principal Investigator: AKSOY, SERAP PHD
Title: COMPARATIVE WOLBACHIA GENOME SEQUENCING
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 2000/04/01-2002/09/30

The objective of this grant is to sequence the genomes of three related Wolbachia strains. These sequences will be determined using a bottom up shotgun approach, assembled, annotated and released to the worldwide scientific community through a searchable internet interface. Three strains of Wolbachia have been selected for sequencing based on their phylogenetic and phenotypic diversity, as well as their presence in human parasitic nematodes and insect disease vectors. The sequencing of three genomes will facilitate a comparative genomic approach to understanding the genetic basis of traits shared by all strains (e.g. intracellular life style) as well as traits unique to each strain (e.g. obligate mutualism in nematodes, versus facultative parasitism in insects, as well as specific reproductive host phenotypes). The data generated from this project will form a framework for future studies which will seek to utilize these agents as targets for the control of human filarial disease, for the control of insect transmitted diseases such as dengue fever and African trypanosomiasis, and for understanding the basic biology underlying their unique interactions with the invertebrate hosts they infect.

Grant: 1U01AI049034-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: HEIDELBERG, JOHN F PHD
Title: WHOLE GENOME SEQUENCING OF COXIELLA BURNETTI
Institution: INSTITUTE FOR GENOMIC RESEARCH ROCKVILLE, MD
Project Period: 2000/09/01-2002/05/31

The zoonotic disease Q fever is caused by the obligate intracellular bacterium *Coxiella burnetii*. *Coxiella burnetii* has evolved strategies to survive in the hostile phagolysosomal environment. This agent is important for developing a comparative perspective on bacterial pathogenesis strategies. Most pathogens either do not survive extended periods in the environment or, like many Gram-positive agents, have evolved the ability to sporulate to survive long periods of desiccation. *Coxiella burnetii* is a Gram-negative organism that has developed a novel, non-sporulating strategy to survive in a non-replicative form for long periods. This property has led to the consideration of this pathogen as a bio-warfare agent. Its stability in the environment presents a serious concern for officials responsible for planning a response to the illegitimate release of this agent. Elucidation of the complete genomic sequence would provide a major breakthrough in the design of therapeutic intervention and a unique perspective on its biology. We propose to sequence the 2.1 Mbp genome of *Coxiella burnetii* Nine Mile, phase I (RSA 493) because of its known virulence potential and subsequent potential for comparison with other isolate groups. The genome sequence will be accomplished by the whole genome random shotgun sequencing currently used at TIGR. Additionally, the genome will be annotated by computer techniques to identify all open reading frames (ORFs) and relate as many ORFs as possible to proteins of known function.

Grant: 1U01AI049036-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: PAULSEN, IAN T PHD
Title: COMPLETE GENOME SEQUENCING OF BRUCELLA SUIS
Institution: INSTITUTE FOR GENOMIC RESEARCH ROCKVILLE, MD
Project Period: 2000/09/01-2003/05/31

The goal of this project is to determine the complete genome sequence of *Brucella suis*, a major cause of the disease brucellosis in humans and food animals worldwide and a significant bioterrorism threat towards both people and food supplies. *B. suis* infection occurs via inhalation, consumption of infected foodstuffs or direct contact with animals and causes debilitating or life threatening disease. Since *B. suis* is easily disseminated by aerial release, it represents a serious biowarfare threat as evidenced by the fact it previously formed part of the now-discontinued US biological weapons research program. Diagnosis of *B. suis* infections is problematic as the initial symptoms mimic those of other diseases, antibiotic therapy is frequently ineffective in the short term due to the intracellular lifestyle of the organism, and no effective *B. suis* vaccine has been developed. The molecular basis of *B. suis* pathogenicity and virulence remains poorly understood. The specific aims of this proposal are therefore to: determine the complete nucleotide sequence of the 3.25 Mb genome of *B. suis*, annotate and analyze the genome sequence, and to make the sequence and analysis of the genome available to the scientific community. The sequencing approach used will be the whole genome random sequencing strategy pioneered by TIGR. TIGR's experience and infrastructure in sequencing, assembly and annotation will ensure that the sequencing of this organism will provide the highest quality sequence at the lowest possible cost. Elucidation of the complete *B. suis* sequence will be crucial for the success of further studies on *B. suis* pathogenicity, and the development of diagnostics, vaccines and therapies.

Grant: 1U01AI049040-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: WALKER, DAVID H
Title: COMPLETE GENOME SEQUENCE OF RICKETTSIA TYPHI
Institution: UNIVERSITY OF TEXAS MEDICAL BR GALVESTON, TX
GALVESTON
Project Period: 2000/08/15-2002/07/31

The complete DNA sequences of the genome of *Rickettsia typhi* will be determined and analyzed for putative coding regions and possible functions of the gene products. Under an addendum for potential bioterrorism agents of the Policy on Support of Large- Scale Genome Sequencing Projects, *R. typhi* has been designated as a priority bioterrorism agent. This application is prepared in response to this opportunity for support of large-scale genome sequencing of high priority pathogens as potential agents of bioterrorism. *Rickettsia typhi* is highly infectious, including via aerosol exposure, infection is deceptively difficult to diagnose clinically and causes a severe, incapacitating illness with 1 percent overall mortality. Knowledge of its genome would be most useful for comparison with that of the more virulent *R. prowazekii* and for investigation of virulence factors, targets of attenuation, and potential novel targets for antimicrobial therapy of strains that could be engineered to resistance to the only two effective agents, tetracyclines and chloramphenicol. Once the genomic sequences are determined and analyzed they will be made available to the research community through an online database with a number of tools to facilitate data retrieval. During the course of the project there will also be ongoing release of preliminary data.

Grant: 1UC1AI049500-01
Program Director: GOTTLIEB, MICHAEL
Principal Investigator: KNIRSCH, CHARLES
Title: Azithromycin Combination Therapy in Resistant Malaria
Institution: CHARLES KNIRSCH, MD, MPH NEW YORK, NY
Project Period: 2000/09/30-2005/03/31

The emergence of in vivo resistance of Plasmodium falciparum malaria to standard antimalarial drugs is a major and growing threat to public health in Africa, Asia, and Latin America. Chloroquine treatment is now rarely effective. The usual replacement, Fansidar, is already losing efficacy. Compliance with quinine can only be achieved for 3 days because of intolerance, and 3 days does not result in cure. Artesunate, also usually given for 3 days, is again not curative. Pregnant women and young children bear the brunt of this disease, and constitute a particularly difficult treatment problem because the drugs commonly added to the above standard agents to augment efficacy-such as doxycycline/tetracycline or mefloquine-are contraindicated for these populations. Azithromycin is an agent that can be safely administered to all the above clinical populations, and the single-agent efficacy of azithromycin in the clinic is comparable to that of doxycycline /tetracycline. The overall aims of this project are to determine if combinations of azithromycin with the above standard agents provide satisfactory efficacy and tolerance including in pregnant women and children and if so, to formally develop such combinations for use in the US and worldwide. B. Specific Milestones and their associated hypotheses MILESTONE 1: test the hypothesis that in phase II "proof of concept"/dose-ranging clinical trials, One or more of the combinations of azithromycin with quinine, fansidar, chloroquine, or artesunate will be effective (90% cure) and well-tolerated for the treatment of uncomplicated P falciparum disease in relatively non-immune populations and also in semi-immune pregnant women. MILESTONE 2: test the hypothesis that in phase II/III clinical trials, the efficacious azithromycin combinations identified in milestone 1 are as safe and well tolerated. MILESTONE 3: test the hypothesis that in phase III pivotal trials including pregnant women and children, the efficacious azithromycin combinations identified in milestones 1 and 2 are safe and effective (95% cure) for drug-resistant malaria. MILESTONE 4: file a supplemental New Drug Application to the United States Food and Drug Administration for the use of azithromycin drug combinations for the treatment of P falciparum malaria, including in pregnant women and children.

Grant: 1UC1AI049520-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: DUNCAN, KEN
Title: Thiolactomycin Acid Derivatives as Novel Antibacterials
Institution: GLAXOSMITHKLINE COLLEGEVILLE, PA
Project Period: 2000/09/30-2005/09/29

The prokaryotic fatty acid biosynthesis pathway constitutes a potentially rich source of essential and novel targets for selective antibacterial drug development. The overall aim of this proposal is to develop a novel class of orally bioavailable, developable preclinical lead molecules that are potent and specific inhibitors of bacterial b-ketoacyl-ACP synthases and possess antibacterial activity against multi-drug resistant Mycobacterium tuberculosis (MDR-TB), Staphylococcus aureus (MRSA) and enterococci (VRE). These novel antibiotics will be obtained by the construction of focused combinatorial chemical libraries designed using the three-dimensional structure of FabB complexed with the thiotetronic acid thiolactomycin (TLM). Thiolactomycin is an excellent candidate upon which to base an optimization strategy. In vitro and in vivo antibacterial activity have already been demonstrated for TLM against the target organisms and it has been shown to have physicochemical properties compatible with being orally bioavailable and non-toxic. In order to achieve the required therapeutic product profile, improvements in biological activity are required and we have demonstrated numerous times that these can be achieved by intensive combinatorial and array chemistry in combination with structure-based design. Thiolactomycin has never been the subject of such a medicinal chemistry program. b-ketoacyl-ACP synthases will be cloned, expressed, purified and crystallized from key pathogens, including the above, and assays configured to permit high throughput screening of TLM analogues. Potent leads will be the subject of state-of-the-art mechanistic enzymology, cocrystallography studies to drive SAR, in vitro and in vivo antibacterial testing and preliminary pharmacokinetic analysis to maximize the generation and optimization of genuine drug candidates. An added advantage accrues because such drugs would be insensitive to current major antibiotic resistance mechanisms. Bacterial type b-ketoacyl-ACP synthases are also present in malaria and other Apicomplexan parasites, as well as Trypanosomes. There is a distinct possibility that these serious pathogens may be included in the spectrum of activity of novel TLM analogues. SmithKline Beecham has a world-leading antibiotic franchise and is uniquely placed to drive the development of novel classes of antibacterial drugs.

Grant: 2R01AR040312-11
Program Director: MOSHELL, ALAN N.
Principal Investigator: MODLIN, ROBERT L MD
Title: CD1-RESTRICTED T-CELL RESPONSES IN SKIN
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 1991/04/01-2005/05/31

DESCRIPTION: (Adapted from the applicant's abstract) - Human CD1 comprises a family of antigen presentation molecules with a unique tissue distribution that includes skin. Experiments performed in the principal investigator's laboratory indicate that CD1-restricted T cells recognize lipid antigens and can contribute to cell-mediated immunity in leprosy. In this application, the principal investigator proposes to explore the immunobiology of antigen presentation by CD1 in human disease, by investigating the cutaneous lesions of leprosy. The Specific Aims are as follows: 1) The structure of antigens presented by human CD1 molecules will be elucidated by deriving CD1-restricted T cells from both tuberculoid and lepromatous leprosy lesions. The principal investigator proposes to determine whether distinct lipid antigens and/or CD1 restricting elements differentially contribute to the host response to infection. 2) The critical antigen-binding sites for lipid and lipoglycan antigens on the molecular surface of the ligand-binding groove of CD1b will be mapped by engineering mutant CD1 molecules that can be used in antigen-presentation and antigen-binding studies. 3) The functional role of CD1-restricted T cells in skin will be investigated by comparing CD1-restricted T cells from tuberculoid vs. lepromatous lesions according to the pattern of secreted cytokines, cytolytic and antimicrobial activity, and their ability to provide help for B cells in the production of antibodies. The studies proposed are intended to provide a comprehensive and in-depth view of CD1-restricted T cell function in relation to a model of human skin disease, with particular emphasis on their role in immunity. Such insights would provide new avenues for development of new treatments for a variety of human skin and infectious diseases.

Grant: 2R01AR041256-09
Program Director: MOSHELL, ALAN N.
Principal Investigator: LEUNG, DONALD Y MD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: THE ROLE OF BACTERIAL TOXINS IN HUMAN SKIN DISEASE
Institution: NATIONAL JEWISH MEDICAL & RES CTR DENVER, CO
Project Period: 1992/07/17-2005/05/31

Atopic dermatitis (AD) and psoriasis are the two most common chronic inflammatory skin diseases in the general population. Colonization and infection with *S. aureus* has been reported to exacerbate AD and psoriasis. The mechanisms by which bacteria participate in the pathogenesis of these skin diseases are unknown. Recent studies demonstrating that approximately 60% of *Staphylococcus aureus* from AD and psoriasis patients produce superantigens (SAGs) provide a plausible mechanism by which *S. aureus* could exacerbate skin inflammation. In particular, it has been shown that staphylococcal SAGs can engage HLA-DR on macrophages and activated keratinocytes to induce the release of cytokines and cause the selective stimulation of T cells expressing specific T cell receptor (TCR) V β regions. Indeed in AD, SAG production has been associated with more severe skin disease. *S. aureus*, which do not secrete SAGs, produce alpha toxin, a potent keratinocyte activator in vitro whose effects on the immune response in vivo is unknown. The specific aims of this competing renewal grant application will be: First, to determine whether AD and psoriasis skin lesions and their respective peripheral blood skin homing receptor positive T cells are associated with a selective expansion of T cells expressing TCR V β regions that react with SAGs on lesional skin. Second, to investigate whether SAGs contribute to the severity of AD by inducing glucocorticoid insensitivity in skin homing T cells, and to assess the mechanisms by which this occurs. Third, to determine the histologic and immunologic effects of staphylococcal alpha toxin vs SAGs on the skin of normal controls vs patients with AD or psoriasis. Genetically-engineered mutant SAGs incapable of binding to either HLA-DR or the TCR will be used to decipher the molecular mechanisms of SAG-mediated skin inflammation in vivo. Fourth, to investigate the mechanisms leading to enhanced colonization of *S. aureus* on the skin of patients with AD and psoriasis. Mutant *S. aureus* selectively deficient in various adhesin genes will be used to define the precise molecules involved in the attachment of *S. aureus* to inflamed skin surfaces. The role of bacterial toxins in the pathogenesis of skin diseases are poorly understood. The skin is an important model to study the pathogenesis of immunologic reactions in tissues. Thus, the elucidation of immune mechanisms by which SAGs exacerbate AD and psoriasis should have important consequences for the development of effective therapeutic modalities in the treatment of a variety of inflammatory diseases. With the increased prevalence of antibiotic resistant *S. aureus* and drug allergy, it is essential to develop new non-antibiotic strategies in combating bacterial toxin-mediated skin diseases.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2R01AR041511-09A1
Program Director: SERRATE-SZTEIN, SUSANA
Principal Investigator: SCHWARTZ, IRA S
Title: NUCLEIC ACID-BASED DIAGNOSTIC PROBES FOR LYME DISEASE
Institution: NEW YORK MEDICAL COLLEGE VALHALLA, NY
Project Period: 1991/09/30-2005/06/30

DESCRIPTION (Adapted from the Applicant's Abstract): Lyme disease is the most common vector-borne disease in the United States. It is caused by the bite of a tick infected with the spirochete, *Borrelia burgdorferi*, the etiologic agent of the disease. Evaluation of the potential for development of disease from a tick bite is complicated by incomplete knowledge of the diversity of spirochete genotypes in nature and their pathogenic capacities. Furthermore, current laboratory diagnosis rests on a number of serologic tests of varying degrees of sensitivity and reliability, which limits the rapid and specific diagnosis of the disease immediately following a tick bite. During the previous grant period significant progress was made in the molecular typing of *B. burgdorferi* clinical isolates. In particular, the investigators observed an unequal distribution of genotypes between skin and blood; a particular genotype which is readily cultured from skin was significantly less frequently found in blood. This suggests that the capacity for hematogenous dissemination may vary among different *B. burgdorferi* genotypes. In addition, progress in the application of a polymerase chain reaction (PCR)-based assay for detection of *B. burgdorferi* in skin, blood, and synovial fluid was achieved. The investigators propose to extend these findings by pursuing the following specific aims: 1) They hypothesize that there is significant genotypic diversity among *B. burgdorferi* in nature, with the greatest diversity in wildlife hosts and the least in human patients. Molecular typing of *B. burgdorferi* in ticks and various wildlife hosts will be carried out by PCR-RFLP analysis and DNA sequencing and the number and distribution of genotypes in reservoir hosts, ticks and patients will be compared. 2) Dissemination of different RFLP types will be tested directly in a murine model. *I. scapularis* colonies infected with single, clonal *B. burgdorferi* genotypes will be established and potential phenotypic variation will be explored with regard to dissemination, acquisition and transmission. 3) The potential of PCR as a modality for diagnosis of early Lyme disease and Lyme arthritis, particularly in patient blood and synovial fluid specimens, will be further explored. A number of variables will be systematically evaluated with the goal of designing a PCR-based approach which can be effectively employed for reliable diagnosis of Lyme disease. The proposed experiments will provide for a more complete understanding of the infection dynamics of *B. burgdorferi*. This will contribute to a better appreciation of the risk of contracting Lyme disease from a tick bite. In addition, a reliable PCR-based method for detection of *B. burgdorferi* in patient blood and synovial should improve accurate diagnosis of early Lyme disease and Lyme arthritis.

Grant: 2R01AR043769-05
Program Director: PANAGIS, JAMES S.
Principal Investigator: GREENFIELD, EDWARD M PHD
Title: CELLULAR MECHANISMS OF IMPLANT LOOSENING
Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH
Project Period: 1996/09/27-2004/08/31

Aseptic loosening of joint implants is one of the major problems in clinical orthopaedics. We and others have recently provided evidence that adherent endotoxin is involved in the stimulation by wear particles of both cytokine production and osteoclast differentiation. However, the importance of endotoxin in vivo is less well studied. Our proposed studies are, therefore, designed to more conclusively test the over-all hypothesis that adherent endotoxin is an important stimulator of wear particle- induced osteolysis. This hypothesis does not suggest that adherent endotoxin is the only stimulator of osteolysis, only that endotoxin is an important stimulator. The following specific aims are proposed: Aim 1. To determine the mechanism of action of adherent endotoxin in vitro. This aim will test three alternative, but not mutually-exclusive, hypotheses. The first hypothesis is that the wear particles deliver endotoxin to the responding cells. The second hypothesis is that adherent endotoxin on the wear particles increases attachment to, and/or phagocytosis by, the responding cells. The third hypothesis is that adherent endotoxin alters the nature of the cellular response to the wear particles. Aim 2. To determine whether adherent endotoxin is an important stimulator of particle-induced osteolysis in vivo. This aim will test two complementary hypotheses. The first hypothesis is that adherent endotoxin is an important stimulator of wear particle-induced osteolysis in mice. The second hypothesis is that wear particles retrieved from patients with aseptic loosening contain substantial amounts of adherent endotoxin. Aim 3. To determine whether adherent endotoxin is an important inhibitor of bone formation on orthopaedic implant materials in vitro. This aim will test two complementary hypotheses. The first hypothesis is that removal of adherent endotoxin will increase attachment, proliferation, and/or osteoblastic differentiation of mesenchymal precursor cells cultured on titanium discs. The second hypothesis is that endotoxin hyporesponsiveness will increase attachment, proliferation, and/or osteoblastic differentiation of mesenchymal precursor cells cultured on titanium discs.

Grant: 2R01AR044252-04
Program Director: SERRATE-SZTEIN, SUSANA
Principal Investigator: DYBVIG, KEVIN F PHD
Title: MECHANISMS OF MYCOPLASMA-INDUCED ARTHRITIS
Institution: UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL
BIRMINGHAM
Project Period: 1997/04/01-2003/03/31

DESCRIPTION (applicant's abstract): Mycoplasma arthritis causes a naturally-occurring, migratory polyarthritis in rodents that bears a close histological resemblance to rheumatoid arthritis of humans. M. arthritis-induced arthritis has been extensively studied as a model for arthritides caused by infectious agents and also as a model for examining the role(s) of superantigens in the development of autoimmunity. All strains of M. arthritis are thought to produce the superantigen MAM, but many an MAM must be required for the development of arthritis. One of these factors is the lysogenic bacteriophage MAV1. Avirulent strains of M. arthritis become virulent when lysogenized with MAV1. MAV1 DNA integrates into the M. arthritis chromosome at any of numerous sites, and the site of integration does not correlate with virulence. Therefore, the increase in virulence associated with MAV1 does not result from changes in regulation of chromosomal genes flanking MAV1 DNA inserts. We have proposed that MAV1 encodes a determinant that is involved with the development of arthritis. MAV1 is the first factor from any mycoplasma that has been shown to be associated with arthritis, and elucidation of this factor is important for fulfillment of the long-range goals of understanding the mechanisms of mycoplasma-induced arthritis and the role of phages as carriers of bacterial arthritogenic determinants. Factors analogous to the MAV1-encoded determinant may be prevalent in bacteria and mycoplasmas that cause arthritis in humans, and these factors may be important as vaccine candidates and as targets for drug design. The goals of the present application are to identify and characterize the MAV1-encoded determinant and initiate studies to elucidate its function. From the nucleotide sequence of the 16-kb MAV1 genome, we have identified a candidate virulence determinant that is predicted to encode a membrane lipoprotein. Specific Aim 1 is to conclusively identify the particular MAV1 gene(s) associated with virulence of M. arthritis. Specific Aim 2 is to determine whether the MAV1 virulence factor is a cytoplasmic or membrane protein and is produced in vivo. Specific Aim 3 is to explore the role of MAV1 in disease pathogenesis. How lysogenization of M. arthritis by MAV1 affects the progression of arthritic disease will be examined. Through the use of immunocompromised animals, we will address the question of whether MAV1 contributes to the virulence of M. arthritis by affecting interactions with host factors such as B and T cells and complement. Thus, the process of dissecting the function of MAV1 in virulence will be begun.

Grant: 1R01AR045408-01A2
Program Director: MOSHELL, ALAN N.
Principal Investigator: REARDON, CHRISTOPHER L PHD
Title: MECHANISMS FOR KERATINOCYTE LIPOPOLYSACCHARIDE RESPONSES
Institution: UNIVERSITY OF COLORADO DENVER/HSC AURORA, CO
AURORA
Project Period: 2000/09/30-2003/08/31

Through a unique form of innate immunity, keratinocytes from humans and mice respond to the bacterial product, lipopolysaccharide (LPS), present in the cell walls of Gram-negative bacteria by secreting inflammatory mediators. The overall objective of the proposed research herein is to define the keratinocyte LPS receptors, LPS-associated functions and signaling mechanisms that occur in this innate keratinocyte anti-bacterial defense system so that patients, who develop a functional loss of this skin-defense system leading up to recurrent bacterial skin infections, may be helped to avoid progressions to bacterial cellulitis and sepsis. We propose: (1) To identify LPS receptors on human and murine keratinocytes in vitro, in situ and in vivo and to determine what agents modulate their expression. Preliminary data shown that the membrane-bound LPS receptors, CD143, CD11a/CD18, CD11b/CD18 and CD11c/CD18 are expressed on human and mouse keratinocytes similar to macrophages. We will use primary keratinocytes and cell lines to further characterize and define the control of the expression of various LPS receptors with several agents and cytokines. We will induce and modulate the expression of LPS receptors in skin organ cultures and in live skin. Keratinocytes from different LPS receptor knock-out mice will be used for comparison. (2) To characterize cellular functions induced by LPS activation of human and murine keratinocytes by determining what cytokines and inflammatory mediators are specifically made in response to bacterial product and to define how these functions can be controlled with agents that modulate the LPS receptors or affect their interactions with LPS. With various methods, we will define the array of inflammatory cytokines and mediators, such as nitric oxide, that are produced following keratinocyte activation with LPS. Keratinocyte functions will be monitored following cell treatments that affect LPS receptor expression to help evaluate which LPS receptors are the most important for the gene promoter transcriptional activators, NF- κ B and NF-IL-6, and up-regulation of CD14-associated type-2 Toll-like receptors following LPS receptor triggering in human and murine keratinocytes and how these events can be controlled through LPS receptor modulation. Cell activation by LPS leads to induction of calcium mobilization across the cell membrane, up-regulation of Toll-like receptor 2 (TLR2) molecules which trigger nuclear translocation of transcriptional activators, such as NF- κ B and NF-IL-6, which in turn bind cytokine gene promoters. We will attempt to show with gel-shift assays, Western blots and quantitative RT-PCR that these signaling agents play an important role in LPS-induced activation of keratinocytes.

Grant: 1R03AR046878-01
Program Director: SERRATE-SZTEIN, SUSANA
Principal Investigator: NARASIMHAN, SUKANYA PHD
Title: BORRELIA BURGENDORFERI GENE EXPRESSION IN TICK MIDGUT--ROL
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 2000/04/01-2003/03/31

DESCRIPTION (Taken from the application): The research application aims to study the gene expression pattern of the spirochete *Borrelia* in the Ixodes tick vector and to comprehend the molecular changes that occur on the spirochetes to facilitate disease transmission. Towards this goal, a novel approach will be utilized to study differential gene expression of tick-adapted spirochetes. Experiments will be carried out to: A) identify genes expressed by the spirochetes at specific feeding time points; B) analyze the temporal and spatial pattern of expression of these genes in an effort to focus on molecules that would be biologically relevant to transmission of disease; and C) to generate mutant *Borrelia* lacking specific genes of interest identified in this application and test the potential of these mutant *Borrelia* to transmit disease. The protein products of the genes that are inferred to have a physiological role in disease transmission will ultimately be tested as transmission blocking vaccines. The results of this investigation will enable a better understanding of molecules involved in transmission of Lyme disease and permit novel strategies for the intervention of disease. The investigation will also be conducive to the development of a useful molecular tool/approach to analyze the gene expression patterns of other vector-borne pathogens in their vector environment.

Grant: 1R03AR047186-01
Program Director: TYREE, BERNADETTE
Principal Investigator: GERARD, HERVE C PHD
Title: ROLE OF APOE IN THE PATHOGENESIS OF CHLAMYDIA PNEUMONIAE
Institution: WAYNE STATE UNIVERSITY DETROIT, MI
Project Period: 2000/09/01-2003/08/31

DESCRIPTION (Taken from the application): An infectious etiology for reactive arthritis (ReA) has long been suspected since this disease often follows primary infections of the gastrointestinal, urogenital, and respiratory tracts. While many bacterial species have been associated with the disease, the genital pathogen *Chlamydia trachomatis* has emerged as a primary agent due to its high prevalence in the population. However, another species of *Chlamydia*, *Chlamydia pneumoniae* shows even more widespread prevalence than does *C. trachomatis*. *Chlamydia pneumoniae* is a pathogen responsible for various respiratory infections and some reports have associated *C. pneumoniae* with heart diseases and even Alzheimer's disease. Importantly, studies from several groups have provided indirect evidence that this organism may be involved in synovial pathogenesis. Preliminary electron microscopic (EM) and polymerase chain reaction (PCR) studies of synovia from arthritis patients and asymptomatic patients confirmed the presence of *Chlamydia pneumoniae* in human synovia. In the present application, we describe studies to confirm and extend our preliminary observations. We will continue our initial screening of the presence of *Chlamydia pneumoniae* in the synovium of arthritis patients and we will determine whether patients with AD show also synovial *Chlamydia pneumoniae* with/without joint pathology, at a higher rate than that of standard arthritis patients. The molecular genetic, EM, IH and other laboratory methods required for these studies are already developed or in place in the collaborators' laboratory. The studies proposed in this application will confirm the presence of *Chlamydia pneumoniae* in the human synovium, and define metabolic characteristics of the organism and host synovial responses to chlamydial infection in addition to the role of APOE in the pathology of *C. pneumoniae*. Such new information will augment our understanding of the pathogenesis process leading to arthritides.

Grant: 2P01CA016519-26
Program Director: WONG, MAY
Principal Investigator: KELLY, THOMAS J MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC
Title: PROGRAM ON MOLECULAR BIOLOGY OF VIRAL TUMORIGENESIS
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 1975/06/01-2005/03/31

The main objective of this program is to contribute to an understanding of the molecular mechanisms responsible for cancer by investigating fundamental genetic processes involved in the control of cell growth and the maintenance of genomic stability. The projects that make up the Program collectively address a number of general issues relevant to this objective. These include the replication of tumor virus and cellular genomes, the mechanisms of malignant transformation by oncogenes, the control of the cell cycle, the mechanisms of DNA transposition and chromosomal rearrangement, the regulation of chromosome segregation, and the role of telomerase in normal growth and tumorigenesis.

Grant: 2R01CA037831-15
Program Director: OKANO, PAUL
Principal Investigator: DEMPLE, BRUCE F PHD
BIOCHEMISTRY:NUCLEIC
ACID
Title: OXIDATIVE STRESS--CELLULAR RESPONSES AND GENE CONTROL
Institution: HARVARD UNIVERSITY (SCH OF PUBLIC BOSTON, MA
HLTH)
Project Period: 1984/08/01-2005/01/31

Oxidative stress is widespread in biology and associated with aging and carcinogenesis. In response to such stress, cells modulate gene expression to escape damage or repair oxidative injury. Gene expression linked to oxidative stress has been described but few molecular signaling mechanisms are defined. The investigators have shown that *E. coli* SoxR protein is a novel transcription activator triggered by oxidation, or by nitrosylation of its essential iron-sulfur centers. Oxidation or nitrosylation of the SoxR [2Fe-2S] centers transduces signals of cellular exposure to pro-oxidants or nitric oxide (NO) into expression of antioxidant, DNA repair, and other defense genes. In this proposal the investigators wish to define the biological, biochemical, and structural basis for this redox/NO signal transduction and response. They will explore biochemically and genetically the cellular systems that switch off activated SoxR by reduction, or processing of the nitrosylated form. The reactivity and control of SoxR will be related to biochemical and physical analysis of SoxR protein structure and the isolation of novel mutant forms of the protein and of stable fragments. The detailed interaction of SoxR with DNA will be explored by *in vitro* methods, and new approaches will be developed to test whether DNA supercoiling can drive transcription activation by SoxR *in vivo* as they have recently shown *in vitro*. They will use a novel nanomechanical device to test explicitly and directly the hypothesis that activated SoxR triggers transcription by localized DNA untwisting. They will investigate *in vitro* whether transcription dislodges SoxR from DNA to allow redox equilibration with the protein in solution. They will test whether NO-related compounds such as nitrosothiols, can also activate SoxR by nitrosylating its iron-sulfur centers. The role of SoxRS-inducible genes that mediate cellular resistance to NO will be identified, as well as novel resistance genes controlled by SoxS and the homologous Rob protein. Using the Rob structure they determined recently, they will test possible activating ligands for this proposed environmental response protein. The knowledge gained from these studies will illuminate approaches to dissecting the mechanisms and roles of redox and NO signal transduction in all organisms.

Date Run: 04/26/05

NIH Extramural Support in Bacteriology Research

Grant: 2R01CA041101-16
Program Director: DUBOIS, RONALD J.
Principal Investigator: BOGER, DALE L PHD
CHEMISTRY:CHEMISTRY-
UNSPEC
Title: BIOLOGICALLY ACTIVE CYCLIC PEPTIDES
Institution: SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA
Project Period: 1985/07/01-2005/04/30

Studies on the total synthesis of the glycopeptide antibiotics vancomycin, teicoplanin, chlorocephalosporins, RP-66453, ramoplanin, and their key partial structures, analogs, and analog libraries are detailed. Their comparative examination may shed further light on the subtle aspects of their site and mechanism of action, identify the role and importance of their structural features, probe engineered structural changes introduced to address the emerging clinical resistance (binding to L-Lys-D-Ala-D-Lac vs L-Lys-D-Ala-D-Ala), and should identify simpler structures with improved properties. Similarly, details of proposed total syntheses of thiocoraline and BE-22179, C2-symmetric cyclic octadepsipeptides possessing two pendant heterocyclic chromophores which are related to sandramycin, the luzopeptins, and quinoxapeptins and which derive their properties through sequence selective DNA bisintercalation, are presented. The definition of the DNA binding properties and biological properties of thiocoraline and BE-22179, key partial structures, and analogs will be conducted as will be a continuation of analogous studies on the luzopeptins and quinoxapeptins. Additionally, plans for the preparation of libraries of analogs of HUN-7293 are detailed to probe and optimize its structural features responsible for inhibition of cell adhesion molecule expression (VCAM-1) and the resultant anti-inflammatory activity and activity in autoimmune diseases.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2R01CA065656-06
Program Director: HALLOCK, YALI
Principal Investigator: HOL, WIM G PHD
Title: STRUCTURE/FUNCTION OF TWO DNA BINDING PROTEINS
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 1995/03/01-2004/12/31

This proposal aims to unravel three dimensional structures of the IdeR family of metal-dependent regulators of topoisomerases I of pathogenic organisms. Also the precise mode of action of a number of inhibitors of human topoisomerase I, called "topo poisons", will be investigated. These include compothecin derivatives recently introduced for the treatment of cancer. In addition, all three-dimensional structures available will be used for the design, synthesis and testing of new topo I poisons of human and pathogenic organisms, and the development of "superactivators" of the IdeR family of regulators. We specifically aim to arrive at agents which modify the action of these proteins in such a manner that the damage to the cellular machinery will be greater than could be expected on the basis of "mere" inhibition or activation of the DNA-binding proteins. This holds both for IdeR superactivators and topoisomerase I poisons which each aim to prolong the lifetime of the protein-DNA complexes targeted thereby involving collisions with replication forks and other entities on the DNA highway, leading to DNA damage and cell death. The four bacterial members of the IdeR family studied differ greatly with respect to DNA sequences recognized and metal ions used for activation. These include Mtb-IdeR from *Mycobacterium tuberculosis*, TroR from *Treponema pallidum*, SirR from *Staphylococcus epidermis* and DtxR from *Corynebacterium diphtheriae*. The human topoisomerase I structures solved recently by our group will initially be the major focus for structure-based inhibitor development. In later stages structures of topoisomerase I from the most important malaria parasite, *Plasmodium falciparum*, and of other major global parasites, will be explored for the development of anti-infectious agents. This project aims at developing better drugs for (i) the treatment of some of the most difficult cancers, (ii) the most important infectious bacterial agent (*M. tuberculosis*), and (iii) the most devastating eukaryotic parasite (*P. falciparum*) known. The latter two account for roughly five million deaths per year worldwide.

Grant: 2R01CA066736-06
Program Director: HALLOCK, YALI
Principal Investigator: KHOSLA, CHAITAN S
Title: ENGINEERED BIOSYNTHESIS OF NOVEL MACROLACTONE POLYKETIDE
Institution: STANFORD UNIVERSITY STANFORD, CA
Project Period: 1995/07/05-2004/04/30

In 1994 this project was initiated based on our ability to functionally transfer the entire 6-deoxyerythronolide B synthase (DEBS) pathway into *Streptomyces coelicolor*, a genetics friendly (and now genomics-friendly) heterologous host. During the past 5 years our efforts have primarily been focused along two directions. On one hand we have sought to develop genetic and biochemical tools to study and manipulate DEBS with the aim of generating new "unnatural" natural products. At the same time, we have conducted a variety of exploratory studies aimed at uncovering novel properties of modular polyketide synthases (PKSs). Over the next 5 years, we propose to continue our twin-track approach of dissecting, at increasing level of detail, the properties of modular PKSs, and developing useful technology for combinatorial biosynthesis. Our plans include (i) the development and application of genetic screens for DEBS mutants with novel properties; (ii) quantification of the incoming acyl chain and the extender unit specificity of individual modules; (iii) elucidating the precise structural features that influence this specificity; (iv) evaluating the feasibility of introducing new chemistry such as methyltransfer into modules; (v) understanding the mechanistic principles underlying vectorial chain transfer between adjacent modules; (vi) elucidating how macrocycle formation is catalyzed, and the molecular recognition features associated with this amazing reaction; and (vii) (if possible) obtaining high-resolution X-ray crystal structures of DEBS and its variants. In the process we expect to refine established strategies for manipulating the intramodular and intermodular chemistry of modular PKSs, and also develop new ones. These next generation methods are likely to expand the molecular diversity attainable by interfacing genetic engineering with chemistry, and also increase the efficiency/yield with which these complex chemicals are made. Given the re-emerging interest in natural product based drug discovery, our long-term goal is to make polyketide natural products increasingly accessible to chemists and biologists alike.

Grant: 2R01CA067985-07
Program Director: OKANO, PAUL
Principal Investigator: DAVID, SHEILA S PHD
Title: RECOGNITION AND REPAIR OF MISMATCHED DNA BY MUTY
Institution: UNIVERSITY OF UTAH SALT LAKE CITY, UT
Project Period: 1995/07/01-2004/06/30

DNA repair has attracted considerable attention due to increasing number of examples linking dysfunctional DNA repair with cancer. In particular, oxidative damage to guanine is emerging as an important causative event leading to mutagenesis and carcinogenesis. Indeed, complex repair pathways for the repair of mutations caused by 7,8-dihydro-8-oxo-2'-deoxyguanosine (OG) have been uncovered. MutY plays an important role in the prevention of mutations by removal of misincorporated adenine residues from OG:A mismatches. MutY belongs to a superfamily of base-excision repair DNA glycosylases and contains a [4Fe-4S] cluster which is more commonly found in electron transfer proteins. Thus, MutY is not only functionally important in maintaining high fidelity DNA replication, it also has unique structural and mechanistic properties. This laboratory has developed a multifaceted research program aimed at providing a detailed understanding of the structural and functional properties of MutY. Our program has initially focused on investigating the basic properties of MutY and clarifying important issues of substrate specificity and chemistry catalyzed by MutY. In addition, we have prepared novel noncleavable substrate analogs for MutY that are effective substrate mimics to characterize the MutY-substrate DNA complex. With this solid understanding of the basic enzymatic properties of MutY, we will turn our attention to investigating in greater detail the properties of MutY involved in damaged DNA recognition and repair. Four specific areas will be examined: (1) We will further characterize the intrinsic chemistry of MutY's recognition and repair of G:A and OG:A mismatches. (2) We will determine the intrinsic properties of G:A and OG:A mismatches that are involved in their recognition and repair by MutY by making specific functional group substitutions on the mismatch or by altering the sequence surrounding the mismatch to determine the recognition elements required by MutY. (3) We will investigate the properties of the MutY-DNA complex using structural and biochemical methods to gain insight into factors involved in damage specific recognition by DNA repair enzymes. (4) We will investigate the role of MutY's [4Fe-4S] cluster loop motif (FCL) in damaged DNA recognition by making specific alterations in amino acids of the FCL and determining the effects on MutY's functional properties.

Grant: 1R01CA079678-01A2
Program Director: HALLOCK, YALI
Principal Investigator: HAYGOOD, MARGO G PHD
Title: ROLE OF BACTERIA IN THE PRODUCTION OF BRYOSTATINS
Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA
Project Period: 2000/05/12-2003/03/31

DESCRIPTION (verbatim from applicant's abstract): In many cases natural products from marine invertebrates are known or suspected to be biosynthesized by bacterial symbionts. The marine bryozoan *Bugula neritina* is the source of the bryostatins, a unique family of compounds with anticancer activity. *B. neritina* harbors a specific bacterial symbiont "*Candidatus Endobugula sertula*." We hypothesize that the biosynthetic source of bryostatins in *B. neritina* is the bacterial symbiont "*Candidatus Endobugula sertula*." Our long-term objectives are: 1) to investigate the role of microbial symbionts in the biosynthesis of marine natural products, 2) to develop methods to exploit symbionts and their biosynthetic genes for economical and environmentally sound production of drugs derived from marine natural products, and 3) to investigate and exploit biosynthetic pathways of marine microorganisms to produce known and novel structures. The specific aims are: 1) to determine the biosynthetic origin of bryostatins in *B. neritina*. 2) To clone the biosynthetic genes for bryostatins in and express them in a heterologous host. 3) To cultivate any bacterial symbionts identified as a source of bryostatins and induce expression of bryostatin production in culture. The bryostatins are complex polyketides, a chemical family that contains many compounds with diverse and potent biological activities. The bryostatins have potential for treatment of cancer and other diseases; bryostatin 1 is in Phase II clinical trials for cancer treatment. The chief obstacle to development of bryostatins is lack of supply. Our project aims to solve this problem by either 1) cloning the genes for the biosynthetic pathway and expressing them in a heterologous host, or 2) cultivating a bryostatin-producing symbiont. The research approach will be to localize bryostatin biosynthesis within the bryozoan, clone polyketide synthase genes, and express them in a *Streptomyces* host. In addition, we will use a novel approach to optimize cultivation conditions to isolate "*E. sertula*" in culture.

Grant: 1R01CA081172-01A1
Program Director: HALLOCK, YALI
Principal Investigator: SHERMAN, DAVID H
Title: BIOCHEMISTRY AND MOLECULAR GENETICS OF MITOMYCIN BIOSYNT
Institution: UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN
Project Period: 2000/01/01-2003/12/31

The overall aim of the proposed research is to understand the molecular mechanisms controlling the biosynthesis of and cellular resistance to the antitumor antibiotic, mitomycin C. This important chemotherapeutic agent is biosynthetically derived from a shikimate pathway metabolite (3-amino-5-hydroxybenzoic acid) and D-glucosamine. In this work, molecular genetic, biochemical and chemical approaches will be used to obtain information on the functional role of the set of genes and enzymes involved in constructing this important anticancer drug. Our initial work demonstrated that *Streptomyces lavendulae* (the mitomycin producer) had at least two genetic loci (*mcr* and *mrd*) that specify resistance to mitomycin. Identification of cosmid clones containing DNA adjacent to the resistance genes revealed that mitomycin biosynthetic genes are clustered around the mitomycin resistance determinant (*mrd*). Using probes for shikimate pathway genes, homologs to the dehydroquinase, dehydroquinase synthase, 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase and 3-amino-5-hydroxybenzoic acid synthase (AHBAS) were identified among a total of 47 genes within the 55 kb cluster. With this information in hand, gene disruption/replacement, mutant complementation, and biochemical experiments will be performed to probe the precise function of the individual mitomycin biosynthetic enzymes. This information will be used to identify and characterize the mechanism and specificity of the enzyme(s) responsible for coupling the AHBA precursor to the D-glucosamine sugar moiety. Subsequently, studies will be initiated to understand and manipulate enzymes involved in establishing the core mitomycin structure and the specificity of tailoring enzymes that provide molecular diversity to this significant class of metabolites. Concurrently, our work will continue on the resistance mechanisms that provide cellular self-protection against mitomycins in the producing organism. Overall, this work will provide an important theoretical and experimental base for future combinatorial biology-based production of novel AHBA-derived natural products using molecular genetic technology.

Grant: 1R01CA082154-01A1
Program Director: YOVANDICH, JASON
Principal Investigator: VALLERA, DANIEL A
Title: RETROVIRAL IMMUNOTOXINS FOR LEUKEMIA
Institution: UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN
Project Period: 2000/04/01-2003/03/31

DESCRIPTION: (Applicant's Abstract) Immunotoxins (IT) are experimental pharmacologic agents that are made by linking antibodies or cytokines that specifically bind to cancer cells to potent catalytic toxins of which a single molecule can kill a cell. The major purpose is to deliver therapy selectively to cancer cells instead of nontarget organs as does conventional chemotherapy. Although these agents selectively bind and kill cancer cells, clinically they have been limited by their 1) failure to penetrate and localize in adequate concentrations in cancer target tissue 2) localization in nontarget organs limiting the tolerated dose and collapsing the therapeutic window. In this application, the applicant will explore a solution to this problem. Cells of the immune system such as T cells are the most prominent cell types that penetrate, attack, and destroy cancer cells and are naturally suited for the expression and production of cytokines in response to antigenic challenge. Therefore, he proposes using T cells to deliver retroviral IT (retIT) consisting of IL-4 spliced to genetically modified diphtheria toxin at the site of the leukemia cells. He has established a model of retIT therapy that he will use as a foundation for future attempts to modify and improve retIT. He will test the usefulness of retIT for therapy of myeloid leukemia, the most common adult form of leukemia. In this model, the applicant has produced an antigen specific CTL cell line called T15 by hyperimmunization with irradiated murine myeloid leukemia C1498. When T15 is transduced with retrovirus encoding IL-4 spliced to truncated DT, T15 cells have been shown to express and secrete IL-4 retIT which specifically kills IL-4R+ C1498 cells, but not IL-4R- cells in vitro. More importantly, mice given C1498 tumors show significantly enhanced anti-C1498 effects when treated with transduced T15 cells as compared to controls. In this application in the first aim, the applicant intends to use this model first to answer important questions regarding the role of IL-4 IT and T15 CTL in the first retroviral model. Is secretion of IL-4 IT necessary and how much must be secreted to get an anti-cancer effect? Does the amount of secreted retIT correlate with the magnitude of the anti-cancer effect? What is the role of the CTL vehicle in the retIT response? Can we enhance secretion and CTL delivery of retIT? In the second aim, he will determine if retIT administration has advantages over conventional IT administration particularly regarding their toxic effects on nontarget organ systems and effects on the immune response. With the anti-C1498 effects that he has already established as a baseline, in the last aim he will ask whether or not important genetic modifications can improve retIT.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01CA082312-01A1
Program Director: HALL, LEOTA
Principal Investigator: DUBOIS, ANDRE T
Title: BACTERIAL & CHEMICAL CARCINOGENS IN GASTRIC ONCOGENESIS
Institution: HENRY M. JACKSON FDN FOR THE ADV ROCKVILLE, MD
MIL/MED
Project Period: 2000/04/01-2004/03/31

Abstract Text Not Available

Grant: 1R01CA084008-01
Program Director: FINERTY, JOHN F
Principal Investigator: MILLER, JEFFERY F
Title: RECOMBINANT LISTERIA VACCINES FOR MELANOMA
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 2000/02/05-2004/01/31

Listeria monocytogenes is a gram positive bacterium that is able to enter host cells, escape from the endocytic vesicle, multiply within the cytoplasm and spread directly from cell-to-cell without encountering the extracellular milieu. The ability to gain access to the host cell cytosol allows proteins secreted by the bacterium to efficiently the MHC class I antigen processing and presentation pathway leading to the induction of CD8+ cytotoxic T cells (CTL). We developed a genetic system for expression and secretion of foreign antigens by L. monocytogenes. Recombinant vaccine strains expressing the lymphocytic choriomeningitis virus (LCMV) nucleoprotein (NP), or a specific MHC class I restricted NP epitope, are able to induce LCMV specific CD8+ cytotoxic T lymphocyte (CTL) responses in mice following vaccination. These strains confer antiviral protection as indicated by the ability of immunized mice to efficiently clear LCMV infection. Listeria strains that express the E11 protein cottontail rabbit papillomavirus (CRP) have also been constructed. Immunization of rabbits with these recombinants causes regression of CRPV induced papillomas and protection from carcinoma. These and other results demonstrate the utility of Listeria vaccine strains for inducing antiviral and anti-tumor immunity. Our objectives are to explore the utility of recombinant Listeria strains as anti-tumor vaccines and determine optimal strategies for attenuation and antigen delivery. The specific aims are to: 1. Construct L. monocytogenes vaccine strains expressing tumor rejection antigens. L. monocytogenes strains expressing the melanoma associated tumor rejection antigens gp100, MART1, TRP2, or an H-2K/b restricted TRP2 181-188 epitope will be constructed. 2. Determine the effects of prophylactic or therapeutic administration of recombinant Listeria vaccine strains on tumor establishment, growth and regression. Two murine tumor models will be used to test vaccine efficacy. 3. Construct Listeria innocua vaccine strains and compare their immunogenicity with wild type and attenuated L. monocytogenes. L. innocua is a non-pathogenic member of the Listeria genus. By transferring genes from L. monocytogenes, we will attempt to construct chimeric strains that are immunogenic yet avirulent.

Grant: 1R01CA084374-01A1
Program Director: HALLOCK, YALI
Principal Investigator: THORSON, JON S PHD
Title: STUDIES ON CALICHEAMICIN BIOSYNTHESIS AND SELF RESISTANC
Institution: SLOAN-KETTERING INSTITUTE FOR NEW YORK, NY
CANCER RES
Project Period: 2000/05/01-2004/04/30

Calicheamicin gammaII (1) from *Micromonospora echinospora* spp. *calichensis* is the most prominent of the enediyne family and its unprecedented molecular architecture in conjunction with its superb biological activity and therapeutic value brand 1 an excellent target for the study of natural product biosynthesis and self-resistance. While the synthetic achievements toward 1 have been monumental, the total synthesis of 1 is secondary to the isolation of 1 from large fermentations of *M. echinospora* and thus, methods to produce mass amounts of 1 and potentially useful variants are still desperately needed. Furthermore, the notably unusual architecture of 1 implies the participation of a remarkably novel biosynthetic pathway. Through a multi-disciplinary approach, the fundamental goal of this proposal is to present rational strategies from which to build a foundation of knowledge regarding 1 biosynthesis and self-resistance. Specifically, we will i) clone the gene cluster encoding for 1 biosynthesis and self-resistance in *Micromonospora*; ii) elucidate the nucleotide sequence of this unparalleled gene cluster; iii) develop a transformation system for *Micromonospora echinospora*; iv) provide functional assignments for the putative genes involved in aryltetrasaccharide assembly; v) localize the specific 1 self-resistance genes within this cluster; and vi) elucidate the mechanism by which the corresponding encoded 1 self-resistance proteins function. Achieving these aims will not only provide pioneering discoveries in mechanistic enzymology (formation of aglycone diynes, hydroxylamine glycosides, thioester sugars, and phenolic-rhamnosides as well as unprecedented modes of resistance to exceptionally reactive natural products), but may also provide access to the rational biosynthetic modification of enediyne structure for new drug leads, biosynthetic methods to introduce 1-targeting or tagging ligands, the potential to construct enediyne overproducing strains and possibly even an enediyne combinatorial biosynthesis program. Should the self-resistance mechanism(s) be applicable, these studies may also lead to gene therapy approaches (via introduction of 1 drug resistance genes into bone marrow cells) for increasing tolerable chemotherapeutic dose levels of 1.

Grant: 2U19CA052955-11
Program Director: HALLOCK, YALI
Principal Investigator: CREWS, PHILLIP O PHD CHEMISTRY, OTHER
Title: MOLECULAR APPROACHES TO DISCOVER MARINE NATURAL PRODUCT
Institution: UNIVERSITY OF CALIFORNIA SANTA CRUZ SANTA CRUZ, CA
Project Period: 1990/09/01-2005/04/30

The broad goal of this proposal, that continues NCPDDG grant CA52955, is to discover marine natural product leads for cancer chemotherapy. We seek structures from microorganisms and cultured microorganisms that will be useful against solid tumors for which there are few effective anti-cancer drug leads. The overall aims are: (a) to show that primary screens aimed at novel targets (oncogene products, tumor suppressor gene products, proteins involved in signal transduction, cell cycle regulation, angiogenesis and apoptosis) will guide the fractionation of natural product mixtures and identify active pure compounds. (b) To demonstrate that murine macro- and microorganisms will provide new anti-cancer leads. (c) To implement pre-fractionation strategies, which will provide an accelerated and more efficient paradigm for the identification of novel, active natural products. (d) To identify compounds in the primary and secondary screens, which act through novel mechanisms. (e) To employ in vivo PK, PD and efficacy studies (subcutaneous or orthotopic human tumor xenografts in nude mice and/or hollow fiber assays) to identify compounds with potential utility against important human solid tumors such as breast, colon, lung, ovarian and prostate. The strategy is to unite novel marine chemistry with unique assays based on new understanding in the molecular biology of cancer. The assays focus on a number of cancer relevant targets associated with the cell cycle, signal transduction, angiogenesis or apoptosis. The primary screens will be employed to examine library compounds and extracts of shallow and deep water murine organisms include: (a) sponges, (b) tunicates, (c) algae, (d) corals, (e) wild cyanophytes, (f) sponges with large populations of cyanophytes, (g) various cultured microalga, (h) cultured actinomycetes, and (i) cultured sponge-derived Gram negative bacteria. Active substances will be isolated, their structures established, and synthetic analogs and derivatives will be prepared for lead optimization. The program design has been effective as one marine natural product compound family of our NCDDG is the subject of a clinical trial scheduled for May 2000. Two other classes are seeds for further advanced development work. The project participants have a strong record of collaborative research and represent different institutions and distinct scientific disciplines: Cancer Biology and Medicinal Chemistry (Novartis Institute of Biomedical Research (NIBR) led by Dr. K.W. Bair; Marine Bioorganic Chemistry (University of California, Santa Cruz (UCSC) led by Dr. P. Crews, continuing as the overall PI; Marine Pharmaceutical Chemistry (Oregon State University (OSU), led by Dr. W.H. Gerwich and Marine Bioorganic Chemistry and Oceanography (Harbor Branch Oceanographic Institution (HBOI) led by Dr. A.E. Wright.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2U19CA067763-06
Program Director: WOLPERT, MARY K
Principal Investigator: PARKER, WILLIAM B
Title: TUMOR SENSITIZATION TO PURINE ANALOGS BY E.COLI PNP
Institution: SOUTHERN RESEARCH INSTITUTE BIRMINGHAM, AL
Project Period: 1995/09/08-2005/04/30

DESCRIPTION (Applicant's Description) The selective expression in tumor cells of non-human genes that can produce toxic drugs is being considered for the treatment of various solid tumors that are refractory to existing chemotherapeutic agents. We have developed a strategy utilizing the substrate characteristics of E. coli purine nucleoside phosphorylase (PNP) to activate purine nucleoside prodrugs (E. coli PNP recognizes adenine-containing nucleosides as substrates whereas human PNP does not). Our approach has numerous characteristics that distinguish it from the other suicide gene therapy strategies (for example, high bystander activity, potency of the toxic purines generated, activity against non-proliferating tumor cells, potent anti-tumor effects after 1 dose of compound). During the last four years we have progressed from a new in vitro observation to successful demonstration of the feasibility of this approach in animal tumor models. We believe that the unique attributes of the E. coli PNP gene therapy strategy in conjunction with some of the existing vectors could result in significant improvements in antitumor therapy. The long-term objective of this application is to develop this idea into a useful anti-tumor therapy. To reach this objective we have three major goals: 1) to demonstrate the feasibility of this approach in animal tumor models; 2) to create better prodrug/enzyme combinations to improve upon the success that we have had with E. coli PNP; and 3. to generate an increased basic understanding of this strategy. This NCDDG is composed of 4 programs and one core. The objective of the: Molecular Biology Program is to develop procedures to selectively deliver genes into tumor cells of whole animals; Biochemistry Program is to fully characterize the biochemical pharmacology of the purine nucleoside analogs and their respective bases to aid in the rational design of new prodrugs; Chemistry Program is to design and synthesize nontoxic purine nucleoside prodrugs that are cleaved to toxic purines by new enzymes developed in this application; X-ray Crystallography Program is to determine the structure of PNP's to aid in the design of new compounds and enzymes; and Chemotherapy Core is to evaluate the antitumor activity of the prodrugs developed in this NCDDG in relevant animal tumor models developed with the aid of the Molecular Biology Program. The overall NCDDG is therefore intended to capitalize on emerging mechanisms for delivery of genes to pre-existing tumors, exciting preclinical efficacy data, and the discovery of novel enzyme/prodrug combinations.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2U19CA072108-05
Program Director: WOLPERT, MARY K
Principal Investigator: JAFFEE, ELIZABETH M MD CLINICAL MEDICAL SCIENCES, OTHER
Title: ANTIGEN-SPECIFIC VACCINES FOR BREAST CANCER
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 2000/09/19-2004/04/30

DESCRIPTION: (Applicant's Description) It is now well accepted that T cells represent a critical immunologic effector in the antitumor immune response produced by therapeutic cancer vaccines. The investigators participating in this NCDDG grant have all been involved in utilizing current molecular technology to develop novel approaches for inducing T cell mediated antitumor immune responses. Candidate tumor antigens have been identified for certain human cancers. An example, which the investigators in our NCDDG program have been evaluating, is the HER-2/neu gene product that is overexpressed in 30% of breast and ovarian cancers. HER-2/neu is one of a large category of recently identified tumor associated non-mutated self-antigens which is overexpressed by tumor cells relative to normal tissue. Although there are many examples of the induction of T cell responses against this category of antigens, currently employed vaccine approaches are not potent enough to overcome the mechanisms of peripheral tolerance that occur to these self-antigens. Therefore, it is necessary to utilize more clinically relevant animal models for identifying the most potent vaccine approaches that can overcome natural mechanisms of tolerance. Dr. Jaffee has been characterizing the immune responses to tumor in the HER-2/neu transgenic mouse model of mammary cancer. In this model, the rat proto-oncogene HER-2/neu, under the control of the MMTV promoter, is overexpressed by spontaneously developing tumors. These mice demonstrate peripheral tolerance to HER-2/neu that is similar to what has been demonstrated in patients with HER-2/neu expressing tumors. Taking advantage of active scientific collaborations which have been ongoing over the past years, the NCDDG group now proposes to utilize this mouse model to evaluate and develop more potent antigen-specific vaccine strategies combined with immune modulators in the form of chemotherapy and biologics, that can overcome tolerance to tumor-associated self antigens. This development will follow a four step process: 1) Individual vaccine strategies under development by the NCDDG project leaders will be optimized for a number of parameters using transplantable HER-2/neu mammary tumors; 2) Baseline immunologic effect functions will be measured as an additional parameter for identifying the most potent vaccine approaches; 3) Optimized vaccine approaches will then be compared head-to-head for the ability to eradicate naturally developing mammary tumors; 4) A targeted evaluation of potential synergies between different vaccine strategies will be evaluated using potency against spontaneous arising tumors as the final outcome.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2R01DC003105-05
Program Director: JOHNSON, THOMAS E
Principal Investigator: DEMARIA, THOMAS F PHD OTHER AREAS
Title: STREPTOCOCCUS PNEUMONIAE ADHERENCE AND OTITIS MEDIA PATH
Institution: OHIO STATE UNIVERSITY COLUMBUS, OH
Project Period: 1996/08/01-2005/07/31

DESCRIPTION: (Adapted from the Investigator's Abstract) Otitis media (middle ear inflammation) ranks first among the most common diagnoses requiring a physician's office visit and recent estimates indicate that virtually all children (99%) will experience a least one episode of otitis media (OM) by age 2. The disease progresses in many children to recurrent infections and chronic inflammation, often with complications and sequelae that include persistent hearing loss and communication disorders. Streptococcus pneumoniae (Spn), is one of the foremost human pathogens and is the primary nasopharynx, the initial event in the induction of OM and the mechanisms which effect the transition for a colonized state to invasion of the middle ear and the induction of the disease state by Spn are not known. The long term objectives of this proposal are to delineate the pathogenic mechanisms involved in Spn adherence, colonization, and invasion of the middle ear and to develop a scientific rationale for the design of novel diagnostic and prevention strategies. The specific aims of this proposal are: 1) To continue to define the role of Spn neuraminidase in the pathogenesis of OM and to assess whether it is a protective antigen and a potential protein-based vaccine candidate, and whether neuraminidase inhibitors are effective in the prevention of Spn nasopharyngeal colonization of OM. 2) To assess the efficacy of lacto-N-neotetraose, a Spn carbohydrate receptor analog, as an anti-infective for nasopharyngeal colonization. 3) To continue to define the mechanisms whereby influenza A virus affects Spn adherence, colonization, and OM. These aims are designed to delineate the initial interaction, adherence, and colonization which represents the first in a series of steps that culminates in otitis media. These studies may suggest avenues for blocking interaction of Spn with host cells either by immunization or direct intervention blockade with isolated receptor moieties or bacterial adhesin components.

Grant: 1R01DC004562-01
Program Director: JOHNSON, THOMAS E
Principal Investigator: LI, JIAN-DONG MD
Title: UP REGULATION OF MUCIN GENE TRANSCRIPTION--OTITIS MEDIA
Institution: HOUSE EAR INSTITUTE LOS ANGELES, CA
Project Period: 2000/07/01-2005/06/30

Otitis media (OM) is the most common childhood infection and also the leading cause of conductive hearing loss in children. While it has been shown that overproduction of mucin, the major protein of mucus in the middle ear, plays an important role in the development of conductive hearing loss, little is known about the molecular mechanisms underlying mucin overproduction. Our long-term goal is to understand the molecular mechanisms by which mucin is up-regulated in OM. Based on our recent studies showing up-regulation of mucin transcription by Gram negative bacterium *Pseudomonas aeruginosa* in cystic fibrosis and that nontypeable *Haemophilus influenzae* (NTHi) is known to be a major Gram negative bacterial pathogen in OM, we hypothesize that NTHi surface molecules up-regulate mucin gene transcription via activation of specific signaling pathways in middle ear epithelial cells. Recent advances in cloning promoter of mucin MUC5AC gene and establishing middle ear epithelial cell lines offer the opportunity to address the proposed hypothesis at molecular and cellular levels. Our preliminary results indicate that NTHi outer membrane proteins (OMPs) up-regulate mucin MUC5AC transcription via activation of a p38 MAP kinase signaling pathway. These encouraging results have thus laid a solid foundation for further investigation of the molecular mechanisms underlying NTHi-induced mucin transcription. Aim 1. Identify bacterial surface molecules responsible for mucin induction by purifying NTHi surface OMPs using chromatography and testing the fractions and proteins for mucin-inducing ability using luciferase assay. Aim 2. Determine the major intracellular signaling pathway required for NTHi-induced mucin transcription by using specific inhibitors and overexpressing dominant-negative mutants. Aim 3. Identify the NTHi response elements in the mucin MUC5AC gene promoter region and the cognate transcription factors by mutagenesis of MUC5AC promoter, using luciferase assay and gel mobility shift assay. Significance: These studies will provide new insights into the molecular pathogenesis of OM and open up new therapeutic targets for inhibition of mucus overproduction to prevent conductive hearing loss and recurrent infection in OM.

Grant: 1P01DE013191-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: GENCO, CAROLINE A PHD
MICROBIOLOGY:MICROBIO
OGY-UNSPEC
Title: MODULATION MOLECULAR PATHOGENESIS IN SYSTEMIC DISEASES
Institution: BOSTON MEDICAL CENTER BOSTON, MA
Project Period: 2000/04/01-2005/03/31

The major focus of this application is to define the molecular mechanisms of host-parasite interactions as it relates to secondary systemic complications of periodontal disease. We propose to examine the response of defined host cells to the periodontal pathogen *Porphyromonas gingivalis*. The sequelae associated with periodontal disease have received considerable attention over the past few years. However, little is known about the specific interactions of *P. gingivalis* with host cells as it relates to cardiovascular disease and diabetes. The goal of this program project is to begin to define the response of host cells to specific *P. gingivalis* components at the molecular level with particular emphasis on these processes in the context of diabetes, cardiovascular disease, and periodontal disease. Project 1 will examine the molecular mechanisms of *P. gingivalis* interactions with human endothelial cells by defining the endothelial cell receptor for fimbriae and the signal transduction events concurrent with *P. gingivalis* infection. Project 2 will examine the role of *P. gingivalis* fimbriae and LPS in leukocyte recruitment, expression of inflammatory mediators and host-derived proteolytic enzymes, the destruction of hard and soft tissue, and the proliferation of *P. gingivalis* in vivo. These will be examined in the context of diabetes using 2 well defined animal models. Project 3 will examine the role of the macrophage response to *P. gingivalis* LPS. The goal of this project will characterize the LPS receptor and Co receptor in normal cells. There are gaps in our knowledge regarding specific details of the interactions between host cells and *P. gingivalis* particularly in diabetes and cardiovascular disease. This study will use novel approaches to increase our understanding of the molecular mechanisms of host parasite interactions and the modulation of these processes in systemic diseases.

Grant: 2R01DE006669-13
Program Director: MANGAN, DENNIS F.
Principal Investigator: CURTISS, ROY I PHD
MICROBIOLOGY:MICROBIO
OGY-UNSPEC
Title: ANTIGEN DELIVERY SYSTEM DESIGN FOR HOST DEFENSE ANALYSIS
Institution: WASHINGTON UNIVERSITY ST. LOUIS, MO
Project Period: 1983/04/01-2005/04/30

DESCRIPTION: (Adapted from the Applicant's Abstract) The investigators propose using attenuated *Salmonella typhimurium* in mice as a model for investigation of 6 different avenues designed to enhance immune responses to foreign antigens expressed by bacterial vectors. The proposed approaches include engineering *Salmonella* to have an "enhanced" RpoS+ phenotype, specifically decreasing immune response to immunodominant *Salmonella* antigens in an attempt to "upregulate" responses against vectored foreign antigens, investigating the influence of attenuating mechanisms on immune responses to heterologous antigens, improve presentation of T-cell epitopes, investigating mechanisms of enhanced antigen delivery via "self-lysing" bacterial vectors or "runaway" plasmids which hyperexpress antigen in vivo, and lastly investigate the immunogenicity of DNA delivered by attenuated *Salmonellae*. The investigators will use heterologous bacterial antigens, and evaluate strains constructed in mice to assess mucosal, humoral and cellular immunity after oral vaccinations. As improved antigen delivery systems are discovered, it is anticipated that they will construct *S. typhi* or *S. paratyphi A* derivatives thereof for eventual clinical studies in humans.

Grant: 2R01DE010467-05A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: LEYS, EUGENE J PHD BIOCHEMISTRY
Title: MOLECULAR & POPULATION GENETICS OF PERIODONTAL PATHOGENS
Institution: OHIO STATE UNIVERSITY COLUMBUS, OH
Project Period: 1995/02/01-2005/06/30

DESCRIPTION (Adapted from the Investigator's Abstract): Chronic periodontitis affects a large fraction of the population. Although microbes are clearly the etiologic agents, no organism or group of organisms has been unequivocally identified as the primary cause of disease. Molecular exploration of several other natural microbial ecosystems has demonstrated a surprising variety of uncultivated organisms. Microscopy studies of oral flora have demonstrated the association of uncultivated spirochetes with several forms of periodontitis, and recent molecular studies have demonstrated their association with RPP. It is likely that pathogenic oral species such as spirochetes or other, less distinctive morphotypes remain undetected. Studies are proposed to determine if uncharacterized or previously unsuspected bacterial species are present in subjects with periodontitis using a molecular approach that circumvents the need for culturing. The bacterial rDNA genes from plaque samples will be amplified with universal primers and cloned. After prescreening to remove common, characterized species, novel clones will be identified by sequencing. Novel or previously unsuspected species will be examined for their association with disease by examining a bank of existing bacterial DNA samples collected from subjects with periodontitis and periodontally healthy controls. The presence and levels of these species will be determined by quantitative, real-time PCR. The same methods will be used to examine the association of 6 previously implicated cultivable species with periodontitis. Comparisons among species will be made. Periodontitis appears to be, at least to some degree, transmissible between close contacts. Consistent with this, the putative periodontal pathogens *P. gingivalis* and *A. actinomycetemcomitans* also appear to be frequently transmitted between close family contacts. Children often harbor these organisms, particularly when parents are colonized. In children the colonization appears to be transient. In contrast, in adults with periodontitis these bacteria appear to be extremely difficult to eradicate. Studies are proposed to examine the acquisition, stability and family transmission of the 4 most strongly disease-associated species. The presence and quantity of each species will be determined by quantitative, real-time PCR.

Grant: 2R01DE010728-06A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: BAKER, PAMELA J PHD
Title: GENETICS OF BACTERIALLY-INDUCED ALVEOLAR BONE LOSS
Institution: BATES COLLEGE LEWISTON, ME
Project Period: 1994/05/01-2003/03/31

DESCRIPTION (adapted from the Investigator's abstract): Periodontal disease among adult humans is a significant public health burden. It is strongly associated with the gram-negative bacterium, *Porphyromonas gingivalis*, yet bacteria alone do not explain population variance in the disease. There is a notable genetic component to both disease incidence and severity. Mouse models have proven extremely valuable in dissecting the pathobiology of various diseases. Modern molecular genetics, including quantitative trait locus (QTL) analysis, is a powerful tool for unraveling the genetic polymorphisms underlying various diseases in the mouse, including susceptibility and resistance to infectious diseases. Large portions of the murine genome are shared with the human genome, so that identification and localization of murine loci have facilitated discovery of their human counterparts. During the current support period, the Principal Investigator has developed a mouse model in which alveolar bone loss is reliably induced in mice by oral infection with *P. gingivalis*. In collaboration with Dr. Derry Roopenian of The Jackson Laboratory, knockout mice with various discrete immunodeficiencies have been used to identify several factors that contribute to bone loss. The Principal Investigator has also found that different strains of immunocompetent mice differ in their susceptibility to bone loss after oral infection. Through F1 crosses and backcross of these mice, we have initial evidence that susceptibility and resistance to *P. gingivalis*- induced alveolar bone loss are heritable traits. It is proposed to study the genetic basis for this susceptibility and resistance. First, the pathophysiological processes that coincide with bone loss in this mouse model will be further characterized and other phenotypic biomarkers will be developed that correlate with bone loss. Second, QTL analysis will then be used to identify chromosomal regions associated with susceptibility and resistance. Together these aims will provide a fuller description of the pathobiology of *P. gingivalis*-induced alveolar bone loss and will allow the Principal Investigator to map loci and alleles involved in susceptibility and resistance. Mouse strains developed in this investigation will be available for others to study. Knowledge of the genetics gained here has a high likelihood of contributing to the identification of candidate genes in humans.

Grant: 2R01DE010963-04A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: BROWN, THOMAS A
Title: ORAL IMMUNIZATION WITH P. GINGIVALIS VIRULENCE ANTIGENS
Institution: UNIVERSITY OF FLORIDA GAINESVILLE, FL
Project Period: 1995/09/30-2005/05/31

DESCRIPTION (adapted from the Investigator's abstract): Immunological control of microbial pathogens through vaccination is an effective way of dealing with many infectious diseases. Live avirulent *Salmonella* have been shown to be safe, effective delivery vehicles for a wide variety of recombinant antigens and induce systemic and mucosal immunity. The Principal Investigator has shown the feasibility of using *Salmonella* to effectively deliver a putative virulence factor HagB from *Porphyromonas gingivalis* resulting in systemic and mucosal immune responses. Many questions remain concerning the basic immunology of live oral vectors. Factors such as recombinant gene expression level and cellular localization can affect the characteristics of the immune response. The ability to control the characteristics of the immune response, the immunogenicity of the foreign antigen and the induction of memory are all desirable goals for the design of any vaccine. To address these questions three Specific Aims are proposed. Aim 1 will construct strains of *S. typhimurium* expressing variable levels of HagB, characterize the effects of expression level on vaccine viability and immunogenicity, evaluate the immune response parameters including the magnitude, duration and antibody isotype distribution in serum, saliva and vaginal washes, and examine the effects on IgG subclass distribution. Aim 2 proposes to construct strains of *S. typhimurium* which express HagB on their surface, to assess the effects of surface expression on membrane integrity, viability, growth rate, and immunogenicity, to evaluate the effects of surface expression on the magnitude, duration and antibody isotype distribution in serum, saliva and vaginal washes and to examine the effects on IgG subclass distribution. Aim 3 will compare differences in priming, boosting and long-term memory induction by strains expressing varying levels of HagB cytoplasmically, or surface localized HagB. In selected experiments, the immune responses and memory induction will be compared using another *P. gingivalis* potential virulence factor, HagA. These studies will aid in the current understanding of the effects of vaccine parameters on immunogenicity, the pattern of the immune response and factors which affect induction of long-term memory and recall.

Grant: 2R01DE011090-06
Program Director: MANGAN, DENNIS F.
Principal Investigator: VICKERMAN, M. MARGARET
Title: REGULATION OF STREPTOCOCCUS GORDONII GLUCOSYLTRANSFERASE
Institution: INDIANA UNIV-PURDUE UNIV AT INDIANAPOLIS, IN
INDIANAPOLIS
Project Period: 1995/09/30-2005/07/31

Early colonizing dental plaque bacteria such as *Streptococcus gordonii*, form the substratum to which later-colonizing microbial species, including potential pathogens, attach in the process of mature plaque development. The *S. gordonii* single glucosyltransferase (GTF) enzyme hydrolyzes sucrose to form glucan polymers that can facilitate bacterial accumulation on surfaces. The level of GTF activity affects the ability of *S. gordonii* cells to colonize surfaces in vitro and therefore, may affect their ability to establish in ecological niches in vivo. Environmental and growth conditions are known to affect *S. gordonii* GTF activity. Expression of the GTF structural gene, *gtfG*, is positively regulated by the upstream determinant, *rgg*. *S. gordonii* undergo a reversible phase variation between high and low levels of GTF activity. Although the molecular basis for GTF phase variation is not known, nucleotide changes or rearrangements in *rgg* or *gtfG* are not necessary; thus distally-located DNA is implicated in the control of *gtfG* expression. The working hypothesis of the proposed studies is that regulation of GTF activity in *S. gordonii* involves complex molecular interactions that may have ecological implications for survival of *S. gordonii* in the oral cavity. The goal of the proposed studies, to identify environmental signals and genetic elements involved in control of *S. gordonii* GTF activity, will be pursued in four specific aims: 1) to characterize the molecular mechanism of *rgg* regulation of *gtfG* through structural and genetic studies; 2) to use reporter genes fusions to identify distally-located *S. gordonii* genes that affect *rgg* and *gtfG* expression; 3) to identify environmental conditions that affect expression of *gtfG* and its regulatory gene(s); 4) to use random arbitrarily primed PCR to identify additional *S. gordonii* genes that show altered levels of expression in response to genetic or environmental factors that affect GTF activity. It is hoped that these studies will provide an understanding of the molecular interactions and conditions that favor or disfavor glucan production by the commensal organism *S. gordonii*. In the long term, such information may provide insights into factors which control, and possibly select for, a healthy oral flora and potentially be implemented in the development of biologically based therapeutic regimens.

Grant: 1R01DE012882-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: MACRINA, FRANCIS L PHD
GENETICS:BIOCHEMICAL/M
LECULAR
Title: STREPTOCOCCUS SANGUIS MICROBIAL GENOME PROJECT
Institution: VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA
Project Period: 2000/07/01-2003/06/30

DESCRIPTION (Adapted from the Investigator's Abstract): The investigators propose to determine the nucleotide sequence of the genome of *Streptococcus sanguis*. This member of the human indigenous oral microflora has long been recognized as a key player in the bacterial colonization of the mouth. It directly binds to oral surfaces and serves as a tether for the attachment of a variety of other oral microorganisms which colonize the tooth surface, form dental plaque, and contribute to the etiology of both caries and periodontal disease. Furthermore, *S. sanguis* has been long recognized as a leading cause of bacterial endocarditis, a disease of high morbidity which is fatal if untreated. Moreover, *S. sanguis* and other viridans streptococci of the mouth are emerging as life-threatening bloodstream pathogens in neutropenic patients. And such infections are being compounded by the increasing frequency with which penicillin resistance is being observed in this group of organisms. New knowledge about this organism could be used in controlling oral microbial colonization so as to minimize or eliminate plaque-related oral diseases. Since the mouth is the source of *S. sanguis* isolates that cause endocarditis and bacteremia, novel controlling strategies also would have an impact on systemic infections. *S. sanguis* genomic data will provide new insights into this organism's lifestyle and virulence properties that cannot be extrapolated from analyzing the genomic data of even closely related species. The investigators believe that the complete genomic structure of *S. sanguis* is certain to lead to the discovery of new genes, insights into their regulation, and an appreciation of their interactions at both the genotypic and phenotypic levels. This, in turn, will provide researchers with new targets for vaccines and rationally designed drugs.

Grant: 1R01DE013094-01A1
Program Director: BRAVEMAN, NORMAN S
Principal Investigator: DESVARIEUX, MOISE MD
Title: ORAL INFECTIONS, CAROTID ATHEROSCLEROSIS AND STROKE
Institution: COLUMBIA UNIVERSITY HEALTH SCIENCES NEW YORK, NY
Project Period: 2000/06/15-2005/05/31

The overall aim of the proposal is to conduct a prospective cohort study to determine and quantify the independent contribution of periodontal infections to the risk of atherosclerosis and vascular disease in a tri-ethnic population. Seizing the opportunity of the initiation of a larger cohort of stroke incidence in a community of Northern Manhattan where Blacks, Whites and Hispanics live together, and utilize the same health care facilities, we have assembled a multidisciplinary team of investigators in the fields of dentistry, medicine, and public health. 1,050 patients will be randomly selected from the community for the first 18 months of the study and followed over a period of 3 years. At baseline, they will undergo a comprehensive periodontal examination assessing clinical, radiologic, microbiologic (species specific DNA probes), immunologic (species-specific antibodies to periodontal pathogens) and inflammatory (GCF cytokine levels and systematic C-reactive protein and fibrinogen levels) parameters in addition to their extensive investigation for vascular risk factors (lipid profile, homocysteine, glucose, EKG, echocardiography...). At baseline and at 3-year follow up, they will also undergo a high resolution B-mode Doppler ultrasound to measure carotid atherosclerosis. All subjects will be re-interviewed every year. Those who report a change in their clinical status suggestive of an outcome event in the period before the 3rd year assessment will be brought in for evaluation. The concurrent measures of local markers of infection and inflammation together with systemic markers of infection and inflammation should help determine which of the infection or the inflammation component of periodontal disease better explains the purported association with atherosclerosis and stroke.

Grant: 1R01DE013100-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: KOLODRUBETZ, DAVID J PHD
Title: SECRETION MECHANISM OF A NOVEL C. RECTUS S-LAYER PROTEIN
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR SAN SAN ANTONIO, TX
ANT
Project Period: 2000/04/01-2003/03/31

Periodontitis is associated with a dramatic shift in the subgingival microflora towards predominantly gram negative organisms. *Campylobacter rectus* is unique among the gram negative periodontal pathogens in its expression of a 150 kd surface-layer (S-layer) protein. These abundant proteins form homogeneous arrays covering the surfaces of numerous bacteria and are required for bacterial pathogenesis. Thus, it is critical that S-layers are properly localized to the cell surface. There are only a few reports on the secretion mechanisms of S-layer proteins. Interestingly, unlike most other S-layer proteins, no amino acids are removed from the *C. rectus* S-layer protein (*crsA*) as it is transported to the cell surface. In addition, a homologue, *crsD*, to one of the three components of type I secretion systems is found in an operon upstream of the *C. rectus crsA* gene. This suggests that *crsA* is transported by a type I pathway. Most excitingly, the *crsD* operon contains another gene, *crsC*, whose protein has no known function. Analysis of the phenotype of a polar *crsC* mutant we constructed suggests that *crsC* is involved in *crsA* secretion, synthesis or degradation. We now propose to exploit the molecular genetic tools we have developed in order to determine the mechanisms used by *C. rectus* to transport its S-layer to the cell surface. It is our hypothesis that the *crsA* protein will be transported by a type I system. Importantly, we hypothesize that *crsC* will have a novel function in the type I secretion pathway perhaps as a chaperone. One goal of this proposal is to identify the *C. rectus* S-layer sequences that direct it to the cell surface using targeted mutagenesis and gene replacement technology. In addition, the genes required for *crsA* transport will be identified by cloning and sequencing the rest of the *crsCD* operon or by transposon mutagenesis. Importantly, the function of each putative transport gene will be characterized by making non-polar mutants. The resulting characterization of the *crsC* mutants will define, for the first time, the function of this novel protein in S-layer metabolism. Finally, the transport mutants will be examined for the possible mis-localization of non-S-layer proteins since there are two reports of type I transport pathways transporting more than one virulence protein.

Grant: 1R01DE013181-01A2
Program Director: MANGAN, DENNIS F.
Principal Investigator: DELISLE, ALLAN L
Title: LYSIS OF CARIOGENIC BACTERIA BY PHAGE-ENCODED ENZYMES
Institution: UNIVERSITY OF MARYLAND BALT PROF SCHOOL BALTIMORE, MD
Project Period: 2000/09/15-2003/08/31

The long-range, health-related goal of this proposal is to develop species-specific, cell wall-hydrolyzing enzymes encoded in the genomes of phages specific for *Streptococcus mutans* and *Actinomyces naeslundii* as new therapeutic treatments for dental caries. *S. mutans* is the primary etiological agent of human enamel caries, whereas *A. naeslundii* (*A. viscosus*), an early colonizer of dental plaque, has long been believed to be involved in gingivitis and root surface (cementum) caries. The major objective of the research proposed herein is to isolate, purify and characterize the enzymes which enable phages specific for these species to lyse their host cells. The lysis genes of two previously studied phages which are specific for *S. mutans* and *A. naeslundii* will be isolated, cloned and sequenced. To accomplish this, the complete genomes of these two phages will be sequenced, which will allow direct PCR subcloning of their holin and endolysin genes and characterization of their respective products. The DNA sequences of these holin/lysin gene pairs will provide information on their regulatory mechanisms and further our knowledge of the evolutionary relatedness of these viral proteins. Comparative analyses of their deduced primary amino acid sequences may also reveal conserved protein domains that are important in determining their structural and functional properties. Additional cloning experiments will be employed to isolate holin genes, and nearby endolysin genes, from these two phages and three additional oral phages, in order to develop a generally applicable method for directly isolating oral phage lysis genes. Phage DNA libraries will be constructed in a phage vector having a defective holin gene, which will allow recombinants expressing oral phage holins to be selected by complementation (plaque formation) of the defect in the phage vector. Inserts will then be sequenced to identify the phage holin genes and primers complementary to the ends of these genes will then be used to sequence, directly from the phage genomes, the adjacent, downstream endolysin genes. Selected endolysin genes will be subcloned, by PCR, from phage genomic DNAs, or from recombinant phage vectors, into expression vectors and introduced into *E. coli* in order to isolate and purify their gene products. The enzymatic activities of these proteins will then be extensively characterized, including determining the specific bonds which they cleave in the cell walls of their respective hosts. Purified preparations of these lytic enzymes might ultimately be used to kill, in a species-specific manner, *S. mutans* and *A. naeslundii* in dental plaque.

Grant: 1R01DE013328-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: GANESHKUMAR, NADARAJAH DDOT DENTISTRY
Title: GENETIC ANALYSIS OF ORAL STREPTOCOCCAL BIOFILM FORMATION
Institution: FORSYTH INSTITUTE BOSTON, MA
Project Period: 2000/04/01-2000/05/31

Initial colonization of tooth surfaces by oral viridans streptococci, including *Streptococcus gordonii*, leads to the eventual formation of biofilms called dental plaque. The most common disease of man, caries and periodontal disease, result from imbalances in the oral microflora, which allow pathogenic species to dominate. Initial plaque formation is characterized by the adhesion of planktonic cells of bacteria such as streptococci to tooth surfaces via specific salivary proteins of the acquired pellicle. Subsequent growth of these initial colonizers and other bacteria on the abiotic surface leads to the formation of dental plaque. Studies have extensively characterized of the initial binding of the planktonic bacteria to saliva-coated/hydroxyapatite surfaces, but the prerequisite signals that trigger the transition from a planktonic to a sessile mode of life and the subsequent accumulation of dental biofilms are poorly understood. It is hypothesized that novel genes are required for initial dental biofilm formation, and identification of such genes and characterization of their expression will be crucial for the development of novel methods of dental plaque control. A simple, but effective method of microbial accumulation on polystyrene surfaces will be used in this study to characterize isolation of biofilm-defective mutants of *S. gordonii* using Tn916 transposon mutagenesis, (2) characterization of biofilm-defective mutants, and (3) cloning and genetic analyses of biofilm genes. These studies of biofilm formation in streptococci will provide valuable information on the initial stages of dental plaque formation. Understanding the mechanisms involved in biofilm formation will be crucial for the development of novel therapeutic strategies to modify the composition of dental biofilm flora towards that found in health.

Grant: 1R01DE013506-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: CALIFANO, JOSEPH V DDS GENERAL DENTISTRY
Title: POST GENOMIC STUDIES OF VIRULENCE IN ORAL PATHOGENS
Institution: VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA
Project Period: 2000/09/21-2003/08/31

DESCRIPTION:(Adapted from the Investigator's Abstract): Epidemiologic, immunologic and clinical studies indict *Porphyromonas gingivalis* as an important pathogen in periodontitis. In contrast to many pathogens, *P. gingivalis* isolates infrequently display genotypic clonality. As many as 100 different clonal types have been found among periodontitis patients. This proposal will explore the possibility that repeated DNA sequences, including insertion sequence (IS) elements, contribute to genetic plasticity in ways that affect pathogenicity and virulence. The Principal Investigator hypothesizes that adaptive changes might include the evasion of immune defenses. At the molecular level, for example, gene expression could be affected by repeated or mobile genetic elements in several ways including transcriptional activation, insertional inactivation, transposition, deletion or gene amplification. The Principal Investigator further hypothesizes that as repeated sequence DNA including IS elements are likely important in gene regulation and genetic diversity in *P. gingivalis*, they will be found in association with virulence genes. Using *P. gingivalis* W83, the Principal Investigator has demonstrated the utility of a method initially described by Ohtsubo et al to isolate repeated DNA sequences carried by prokaryotic genomes. The Principal Investigator proposes to molecularly clone and characterize a diversity of repeated DNA sequences and use them as tools to evaluate positioning of such nucleotide sequences in *P. gingivalis* W83 with special reference to known virulence genes. The lysine gingipain protease (kgp) gene will be used as a paradigm virulence gene in the proposed studies. This information will be extended to non-clonal clinical isolates. In this fashion, the Principal investigator anticipates using this panel of repeated DNA sequences as tools in exploring the genetics of virulence in non-clonal *P. gingivalis* isolates. The gene probes that will emanate from the proposed studies will provide an operational link between the complete nucleotide sequence of strain W83 and the non-clonal pathogens commonly represented by typical isolates of *P. gingivalis*.

Grant: 1R01DE013523-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: HANDFIELD, MARTIN PHD
Title: IN VIVO INDUCED ANTIGEN TECHNOLOGY OF AA IN LJP
Institution: UNIVERSITY OF FLORIDA GAINESVILLE, FL
Project Period: 2000/05/01-2004/04/30

DESCRIPTION (adapted from Investigator's abstract): Considerable evidence implicates *Actinobacillus actinomycetemcomitans* (Aa) as the etiologic agent of localized juvenile periodontitis (LJP). The goal of this application is to develop and test a fundamentally new and improved approach to identify Aa genes which are expressed during in vivo, but not in vitro, growth. Such genes are likely to be important to Aa's ability to cause LJP. The approach, called In Vivo Induced Antigen Technology (IVIAT), is superior to other related technologies because it does not rely on animal models to mimic the growth of the pathogen in humans. IVIAT uses antibodies present in pooled sera from LJP patients as probes to identify the genes of interest. This application has five specific aims. In the first specific aim, pooled antisera from 20 LJP patients will be exhaustively absorbed with in vitro-grown whole cells and cell extracts of Aa strain HK1651. The resulting serum will be used to probe two independent genomic expression libraries of HK1651 in *Escherichia coli* using colony blotting methods. In Specific Aim 2, the cloned DNA inserts in reactive clones will be analyzed for IPTG inducibility and sequenced to determine open reading frames likely to be responsible for expression of the in vivo-induced antigens. In Specific Aim 3, the open reading frames will be subcloned into an appropriate expression vector and at least 5 mg of the expressed protein will be purified by affinity chromatography. The purified proteins will be bound to a matrix support in Specific Aim 4 and used to affinity purify reactive antibodies from the absorbed LJP patient serum. These purified antibodies are used in Specific Aim 5 to definitively prove that the in vivo induced antigen is actually expressed by Aa during its growth in LJP patients. To accomplish this, plaque samples will be collected from first molars of 10 LJP patients. Aa cells in the plaque samples and their expression of the in vivo induced antigen will be demonstrated with differential fluorescence microscopy using a monoclonal antibody specific for Aa and the purified antibodies prepared in Specific Aim 4. The results of these studies are expected to improve our understanding of the pathogenic mechanisms employed by Aa in periodontal disease causation by identifying virulence-associated genes that would not be found by conventional microbiological or biochemical methods. The results should also validate IVIAT as a useful tool for a broader range of human pathogens. In vivo induced antigens discovered using this method are excellent candidates for consideration as potential diagnostic tools, new targets for antimicrobial therapy, or for vaccine design.

Grant: 1R01DE013541-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: RELMAN, DAVID A
Title: MOLECULAR ANALYSIS OF SUBGINGIVAL MICROBIAL DIVERSITY
Institution: STANFORD UNIVERSITY STANFORD, CA
Project Period: 2000/09/01-2004/08/31

DESCRIPTION (Adapted from the Investigator's Abstract): Bacteria are the primary cause of plaque-associated gingivitis and chronic adult periodontitis. It is unclear, however, whether the microorganisms responsible for gingivitis also contribute to the development of adult periodontitis or whether a unique assemblage of microorganisms is responsible for periodontitis. Studies indicate that plaque-associated gingivitis progresses to chronic adult periodontitis in only a subset of individuals. Previous work on this topic has been hampered by the limitations of cultivation-based methods, taxonomic inconsistencies, insufficient microbial characterization and inter-patient heterogeneity. The proposed work relies upon sensitive molecular methods to identify predictive associations between specific bacterial and archaeal species and the onset of adult periodontitis. The short-term objectives are to determine the subgingival microbial markers of gingival health, plaque-associated gingivitis and chronic adult periodontitis. The long-term objectives are to understand oral microbial ecology, to determine its relationship with local and systemic disease, and by so doing, to identify healthy individuals with increased risk of disease who would benefit from early intervention. The Specific Aims of this proposal are: Aim 1. Identify the bacterial and archaeal species within subgingival plaque from healthy sites in periodontitis patients. Phylogenetic analysis of 16S ribosomal DNA sequences and high density DNA microarrays will be used to identify the microbial species composition of subgingival plaque. Aim 2. Identify the bacteria and archaea from plaque-associated gingivitis and chronic adult periodontitis sites using the approach in Aim 1. Aim 3. Quantify differences in bacterial and archaeal contribution to the total microbial population within subgingival plaque associated with healthy gingiva, plaque-associated gingivitis and chronic adult periodontitis. Slot-blot and in situ hybridization methods will be applied with group- and species-specific 16S rDNA probes. Targeted organisms will include those implicated in the development of disease from the work in the above aims. Among the expected long-term benefits of this work will be the identification of organisms that can serve as predictors of intra-oral health and disease, and the development of broadly useful DNA microarray for microbial surveys and diagnostic studies.

Grant: 1R01DE013545-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: PROGULSKE-FOX, ANN
Title: INTERACTIONS BETWEEN ORAL PATHOGENS AND VASCULAR CELLS
Institution: UNIVERSITY OF FLORIDA GAINESVILLE, FL
Project Period: 2000/04/15-2005/03/31

There has been an emerging paradigm shift from the belief that coronary heart disease (CHD) has a purely hereditary/nutritional etiology to the view that CHD may have an infectious etiology. Among the organisms that are suspected to be involved in CHD are pathogens associated with periodontal diseases. Our hypothesis is that invasion of the cells of the arterial wall by certain oral bacteria could represent the injury that either initiates and/or exacerbates the fibroproliferative response of CHD. Thus the aim of this research is to investigate the possible molecular mechanisms involved in the interactions between periodontal pathogens and cardiovascular (CV) tissues with a focus on invasion of the cells of the arterial wall. We have established that strains of *Porphyromonas gingivalis* and *Prevotella intermedia* invade both human coronary artery endothelial cells (HCAEC) and coronary artery smooth muscle cells (CASMC). This application is designed to characterize the invasion of the HCAEC. The specific aims include: 1) Determine the mechanism of cell invasion, 2) Determine the effects of *P. gingivalis* invasion on cardiovascular cells, 3) Use a reporter gene system (IVET) to identify and isolate genes of *P. gingivalis* involved in cell invasion, 4) Characterize the reporter gene-labeled invasion gene products, and 5) Construct isogenic mutants of each of the putative invasion genes and test the isogenic strains in invasion assays.

Grant: 1R01DE013573-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: DALE-CRUNK, BEVERLY A PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: REGULATION OF B-DEFENSIN EXPRESSION IN ORAL EPITHELIA
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2000/07/15-2005/06/30

DESCRIPTION (Adapted from the Applicant's Abstract): Oral epithelia serve as a first line of defense for oral and periodontal health via innate host defense mechanisms. Human gingival epithelial cells (HGECs) express two antimicrobial peptides of the beta-defensin family, human beta-defensin-1 (hBD-1) and -2 (hBD-2) that contribute to innate immune responses. The potential importance of these peptides in oral health and disease susceptibility is only recently beginning to be appreciated. Production of hBD-1 and hBD-2 is stimulated when HGECs are exposed to an oral commensal bacterium, *Fusobacterium nucleatum*. hBD-1 mRNA is also up regulated by exposure to TNF α , an inflammatory mediator, while hBD-2 mRNA is constitutively expressed. These preliminary studies suggest that commensal organisms may be important in maintaining the normal protective barrier function of the oral mucosa in vivo by partial activation of the innate immune system. Several findings or hypotheses are the focus of this project: 1) that expression of beta-defensins is associated with differentiation in oral mucosa; 2) that beta-defensins are regulated by known or novel cell surface receptors, possibly including CD14 and TLRs, 3) that signal transduction via the transcription factor NF κ B is critical for hBD-2 mRNA expression, 4) that the pathway for stimulation may differ from that of other host innate immune responses, such as the chemokine IL-8, 5) that new gene expression may be required. The present proposal uses cell and molecular studies to investigate the regulation of expression of beta-defensins in relation to epithelial differentiation, and to identify cell surface receptors and signaling pathways responsible for their expression in response to the oral commensal organism, *F. nucleatum*. Understanding the signaling pathways and means to enhance peptide expression will have future potential for enhancing antimicrobial peptide expression for prevention and treatment of oral microbial disorders, including periodontal disease, caries, recurrent candidal infections, and oral mucositis.

Grant: 1R01DE013683-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: MARQUIS, ROBERT E
Title: OXIDATIVE STRESS IN ORAL STEPTOCOCCI
Institution: UNIVERSITY OF ROCHESTER ROCHESTER, NY
Project Period: 2000/09/15-2005/07/31

Oxygen metabolism and resultant oxidative damage caused by metabolically generated reactive oxygen species (ROS) are key processes affecting the activities of plaque bacteria. The organisms have developed multiple mechanisms to protect themselves against oxidative damage, including superoxide dismutase, peroxidases, sulfhydryl reductases and DNA repair enzymes. The major source of O₂ for supragingival plaque is air in the mouth, while that for subgingival plaque is crevicular fluid. Since plaque is a thin biofilm, O₂ moves readily into the film to be metabolized, and plaque generally has been found to have residual O₂ levels about 10% that of air-saturated water. However, the important O₂ is the metabolized portion, which then is the source of ROS. In addition, many oral healthcare products contain hydrogen peroxide, which can be radicalized through Fenton reactions to cause oxidative damage. The objectives of this application are to develop a clearer picture of oxidative damage to plaque bacteria, primarily oral streptococci, and to identify through use of genetic mutants the major protective mechanisms. Part of the project has a very practical aim of devising better ways to control oral infectious diseases with an emphasis on caries and use of peroxides. The specific aims are: 1. Determine the roles played by NADH-linked oxidases, peroxidases and glutathione reductase in protection of oral streptococci against hydrogen peroxide and other oxidatively damaging agents, 2. Define the roles that superoxide and superoxide dismutase play in oxidative damage and protection against damage, 3. Elucidate the interactions of transition-metal cations, chelators and fluoride in oxidative damage, including interactions with oxygen- metabolizing and protective enzymes.

Grant: 1R01DE013759-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: WEINSTOCK, GEORGE M PHD
Title: MICROBIAL GENOMICS
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX
HOUSTON
Project Period: 2000/09/21-2002/08/31

The complete DNA sequences of the genomes of *Fusobacterium nucleatum* and *Bacteroides forsythus* will be determined and analyzed for putative coding regions and possible functions of the gene products. These two bacteria are important in oral microbiology including diseases such as periodontitis. They have been targeted for DNA sequence analysis by a strategic planning workshop for oral infectious diseases of the NIDCR. Oral infectious diseases often are polymicrobial in nature and result from disturbances in the microbial ecology of the oral cavity. Understanding this ecology, both in terms of the capabilities of the normal oral flora as well as their interactions, is crucial to diagnosis, prevention, and cure of such diseases. As a first step, elucidating the complete genetic content of important members of the oral flora will provide a wealth of information that can lead to a profoundly deeper picture of the physiology of these microbes, their virulence factors, mechanism of biofilm formation, as well as their other capacities for interaction with the host and each other. Once the genomic sequences are determined and analyzed they will be made available to the research community through an online database with a number of tools to facilitate data retrieval. During the course of the project there will also be ongoing release of preliminary data.

Grant: 1R01DE013819-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: CHU, LIANRUI MD
Title: H2S PRODUCTION AND VIRULENCE OF TREPONEMA DENTICOLA
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR SAN SAN ANTONIO, TX
ANT
Project Period: 2000/09/01-2003/06/30

DESCRIPTION (Adapted from the Investigator's Abstract): A characteristic feature of periodontitis is the existence of a variety of volatile sulfur compounds produced at sites of destructive disease. Hydrogen sulfide (H₂S) is a major component of this family, which is a primary factor in halitosis related to periodontitis, and importantly, can affect host molecules and cells. Previous studies have identified a limited repertoire of oral microorganisms that can produce and survive in microenvironments with large amounts of H₂S; however, the metabolic enzymes involved in the production of H₂S are poorly understood. Substantial literature has identified *T. denticola* as one of the few oral bacteria with the capacity to produce and grow in high levels of H₂S. The Principal Investigator has previously isolated and characterized an enzyme, cystalysin. This enzyme is unique to *T. denticola* and has a substrate specificity for L-cysteine, yielding pyruvate, NH₃, and importantly, H₂S. This proposal develops the general hypothesis that H₂S is a significant effector of host responses in the local microenvironment of the periodontal disease site and, furthermore, that H₂S-forming enzymes should be considered virulence determinants for *T. denticola*. Three specific aims are developed using biochemical, molecular genetic, and cell biologic studies to address this hypothesis: Specific Aim 1: To construct isogenic mutants altered in cystalysin-directed H₂S producing capacity; Specific Aim 2: To biochemically characterize the enzyme pathway(s) involved in H₂S production from glutathione; and, Specific Aim 3: To determine the effects of an H₂S environment created by *T. denticola* on host inflammatory/immune functions. *T. denticola* has been identified as an important member of a specific consortia of microorganisms considered as etiologic in the development and progression of destruction of the periodontium. This proposal is designed to provide critical information related to the production of H₂S by *T. denticola*, and for the first time to delineate the potential functions of H₂S, which could deleteriously affect the homeostasis, maintained by molecules and cells in the periodontium. The outcomes would also be expected to provide an impetus to further target the enzymatic pathways contributing to H₂S production as an innovative intervention strategy.

Grant: 1R01DE013882-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: BRADY, L J BA
Title: IMMUNOMODULATION BY EXOGENOUS STREPTOCOCCAL ANTIBODY
Institution: UNIVERSITY OF FLORIDA GAINESVILLE, FL
Project Period: 2000/04/15-2004/03/31

DESCRIPTION: (adapted from the Investigator's abstract): Systemic immunization with antigen coupled to monoclonal antibody (mAb) has been used by several investigators to increase the number of mAb-producing hybrids against an antigen and to elicit antibodies specific for poorly immunogenic epitopes. This strategy has implications for vaccine design in that protective immunity is not necessarily directed at immunodominant epitopes of pathogens and could well be improved by shifting a humoral response toward subdominant epitopes. To date, no studies have addressed the potential for immunomodulatory activity mediated by mucosally applied mAbs bound to antigen. To test whether mucosal administration of an exogenous antibody directed against a streptococcal surface protein could influence the humoral immune response, BALB/c mice were immunized orally or intranasally with *Streptococcus mutans* alone or *S. mutans* complexed with a mAb directed against the major surface protein P1. *S. mutans* is a major etiologic agent of dental caries and P1 is a promising vaccine antigen. A passively applied anti-P1 mAb has also been reported to prevent recolonization by *S. mutans* in human subjects, months to years after the exogenous mAb is no longer detectable. Results described in this proposal indicated that binding of an anti-P1 mAb to the surface of *S. mutans* prior to mucosal immunization causes significant changes in the subclass distribution and specificity of elicited antibodies. Either of these changes has the potential to alter the protective capacity of a humoral immune response. This information is important from the perspective of identifying anti-P1 mAbs which could be used to shift immunodominance toward a more protective response, in addition to providing a plausible explanation for the extremely long clinical effect reported after "passive" immunization of human subjects with an anti-P1 mAb. In that case, exogenous mAb may have complexed with recolonizing *S. mutans* to modify the adaptive immune response toward one of increased protection. This proposal is designed to screen a panel of twelve well characterized anti-P1 mAbs for immunomodulatory activity in BALB/c mice and to characterize changes in their immune response; to assess the effect of altered murine responses mediated by anti-P1 mAbs on protection against *S. mutans* using *in vitro* and *in vivo* model systems; to directly test serum from mAb-treated human clinical trial patients for changes in their anti-*S. mutans* response; and lastly to elucidate the mechanism by which anti-P1 mAbs may modulate the immune response against *S. mutans*. Such information would be directly relevant to the study of any active or passive mucosal immunization strategy.

Grant: 1R01DE013992-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: WEINBERG, AARON PHD IMMUNOLOGY AND MICROBIOLOGY
Title: C ALBICANS REGULATION BETA-DEFENSINS IN ORAL EPITHELIA
Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH
Project Period: 2000/09/28-2003/07/31

DESCRIPTION: (adapted from the applicant's abstract) Oropharyngeal candidiasis (OPC) is an emerging disorder owing to the prevalence of AIDS, misuse of antibiotics, and host immunosuppression in general. *Candida albicans* is the most common fungal species isolated from OPC lesions. Recent findings show that mucosal epithelial cells synthesize and secrete antibacterial and antifungal agents, belonging to a family of small, cationic peptides. These molecules, human beta-defensins 1 and 2 (hBD-1, hBD-2) are predicted to function as a first line of host defense against microbial pathogenesis. The PI has discovered that these peptides are expressed in normal human gingival epithelial cells and associated with differentiated epithelium of oral tissues. Moreover, they found that the non oral, yet disseminating isolate *C. albicans* strain SC5314 stimulates beta-defensin expression in oral epithelial cells, but a clinical OPC isolate does not. This proposal intends to test hypotheses relevant to oropharyngeal candidiasis emanating from the postulate that oral epithelial cells can be stimulated to produce beta-defensins that protect the host from candidal challenges at the oral mucosal barrier. The objectives of this proposal are (1) to determine beta-defensin expression in oral epithelial cells in response to challenge with OPC derived *C. albicans* isolates, (2) to characterize key virulence factors of *C. albicans* SC5314 and OPC isolates that lead to beta-defensin response, (3) to examine beta-defensin protection against *C. albicans*, and (4) to identify genes in oral epithelial cells associated with *C. albicans* modulation of beta-defensin expression, using microarray technology. The PI hypothesizes that peptide-based antimicrobial defense may be a way in which the gingival epithelium resists invasion of potential pathogens. In light of the frequent adjunctive use of antibiotics and antimycotics in treating oral diseases, with the threat of microbial resistance, investigations into novel eukaryotic peptides, such as beta-defensins, are highly significant and offer the potential for future clinical promise. The PI states that this research direction may be significant in leading to future studies with potential application to oral disorders, therapeutic use, and technology development.

Grant: 1R03DE012969-01A2
Program Director: MANGAN, DENNIS F.
Principal Investigator: TENG, YEN-TUNG A PHD ORAL PATHOLOGY
Title: IDENTIFY ANTIGENIC DETERMINANTS OF HUMAN PERIODONTITIS
Institution: UNIVERSITY OF WESTERN ONTARIO LONDON, ON
Project Period: 2000/06/01-2002/05/31

Human periodontal diseases (e.g., periodontitis) are heterogeneous and result from specific bacteria-host immune interactions. Periodontitis is the major cause of tooth loss in adults and has been recognised as a significant risk factor associated with coronary heart disease, stroke and bacterial pneumonia. The long-term objective of this research program is to identify bacterial antigens important for immune and inflammatory responses in human periodontitis. Engraftment of immunodeficient NOD/SCID mice with human peripheral blood leukocytes (HuPBL) provides an excellent model for studying immune responses to inoculated pathogens. The applicant proposes to use this unique system and a well characterized clinical entity, *Actinobacillus actinomycetemcomitans*:Aa-associated localized juvenile periodontitis (LJP), to study the immune basis of human periodontitis, for which no animal model exists and which, for ethical and practical reasons, cannot be directly studied in humans. The applicant has shown that oral inoculation of live Aa into NOD/SCID mice carrying high levels of (up to 60 percent) HuPBL from periodontitis patients can be achieved. Further, engrafted human leukocytes present in mouse periodontal tissues can functionally respond to Aa challenge. Therefore, microbial antigen-specific immune responses of LJP can be studied in this model. The specific aims of this proposal are: 1) to identify Aa-antigens involved in LJP using a genetic screening approach, and 2) to assess the periodontal immune responsiveness elicited by the identified Aa-antigens in the current animal model. Identification of Aa-antigens will be achieved by screening an Aa genomic-DNA library in transformed *E. coli*. This would allow the expressed Aa-antigens to be captured by patient's antigen-presenting cells which will present and activate the same host's periodontal CD4+T-cells carrying an activation marker for visual identification as probes. The Aa antigens identified will be assessed by *in vitro* T-cell activation (by IL-2) and B-cell IgG antibody (by ELISA) assays for specificity and correlation analyses in LJP and LJP-free subjects. The new information obtained from the proposed studies will provide rationales and hypotheses to investigate the clinical correlates and significance for important bacterial antigens involved in Aa-associated LJP. Further, it will increase our knowledge of host immune-parasite interactions and could eventually lead to the development of new protocols (e.g., Mabs or vaccines) for the treatment of human periodontal diseases. Therefore, the patient's periodontal health will be improved, thereby their complications and health-care costs will be reduced.

Grant: 1R03DE013446-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: FENNO, J CHRISTOPHER PHD MICROBIOLOGY &
MOLEC. GENETICS
Title: OPPA PEPTIDE HOMOLOGUE OF ORAL SPIROCHETES
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2000/04/01-2002/03/31

Surface-associated proteins of *Treponema denticola* mediate interactions between the spirochete and sub-gingival tissues in periodontal diseases. Proteins having direct cytopathic effects have been a primary focus of research. However, very little is known of the mechanisms this peptidolytic and proteolytic organism must utilize for nutrient acquisition, and may utilize in specific signaling pathways or in evasion of host defenses. These processes can be key factors in the development of many infectious diseases. Molecular genetic studies of oral spirochetes are crucial to analysis of spirochete-host interactions in periodontal diseases and contribute to understanding infectious diseases caused by frankly pathogenic spirochetes. The present proposal explores the potential role of *T. denticola* OppA in periodontal pathogenesis. This protein, present in surface extracts of *T. denticola*, is encoded by a highly conserved genetic locus that includes oppA, -B, -C, -D and -F, the components of an ATP-binding cassette (ABC) transporter family involved in pep-tide nutrient uptake and trans- membrane environmental signaling in a wide range of bacteria. Preliminary studies showed that OppA bound soluble host proteins abundant in inflamed subgingival tissue. We hypothesize that OppA binding of host cell proteins contributes to the pathogenesis of organism by (i) accretion of host peptides or proteins to the spirochete surface, resulting in modulation or evasion of host responses; or (ii) peptide transport into the cell for use in metabolic or environmental signaling pathways. This proposal complements studies underway in this laboratory on assembly of membrane complexes comprised of outer membrane porins and proteases, and includes novel approaches to the study of the role of *T. denticola* in periodontal pathogenesis. Recombinant expression systems will be used to investigate the structure and function of OppA and to characterize its interaction with host extracellular matrix and serum components present in the subgingival environment. Molecular genetic analysis of this putative transport system will contribute to the understanding of bacterial interactions with host tissue components in periodontal diseases, as well as to basic knowledge of the biology of pathogenic spirochetes.

Grant: 1R21DE013703-01
Program Director: BRAVEMAN, NORMAN S
Principal Investigator: PINE, CYNTHIA M PHD
Title: MODELS OF HEALTH INEQUALITIES IN CHILDHOOD DENTAL CARIES
Institution: UNIVERSITY OF DUNDEE DUNDEE,
Project Period: 2000/09/19-2002/09/18

This proposal is submitted to RFA-DE-99-002 'International Collaborative Oral Health Research Planning Program'. The long-term aim of this international research consortium is to determine optimum interventions to reduce dental caries in children in disadvantaged communities and minimize the effects of exclusion from health care systems, of ethnic diversity, and health inequalities. Research is lacking in the interactions vertically in the paradigm between molecular and psychosocial impacts within and between ethnically diverse and impoverished children. Three specific aims are to be addressed under this planning grant. First to examine the microflora of ethnically diverse children with and without dental caries and determine whether there are differences in the pattern, the virulence and the behavior to fluoride. Second, to develop, validate and pilot a measure of familial and cultural perceptions and beliefs that contribute to the development of caries experience in children from diverse ethnic and socio-economic groups. Third, to identify the characteristics of children with adverse health service utilization rates and the characteristics of those health services which result in minimum exclusion and more favorable and equitable health outcomes. This consortium comprises 32 scientists from 10 countries from a range of disciplines with data from many research studies, each augmenting any individual country's efforts. There are 5 WHO Collaborating Centres among the consortium and the support of the European Regional Office of WHO is among those given. This consortium is well placed to make a significant impact on this universal childhood illness.

Grant: 1R21DE014040-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: WILSON, CAROLE L PHD
Title: MODULATION OF BETA-DEFENSINS BY MATRILYSIN
Institution: WASHINGTON UNIVERSITY ST. LOUIS, MO
Project Period: 2000/09/01-2002/08/31

DESCRIPTION (adapted from applicant's abstract): Components of the innate immune system comprise the first line of defense against the encroachment of microorganisms at mucosal surfaces. Deficiencies in this system are believed to play a role in diseases such as Cystic Fibrosis, which is characterized by chronic bacterial infection. Defensin peptides are abundant components of the innate immune system, particularly in the human upper airway. Respiratory mucosal epithelium produces human beta-defensin precursors, known as hBD-1 and hBD-2, which undergo amino-terminal processing to attain full antimicrobial activity. However, the mechanism by which this processing occurs is not known. Previous work in mice has demonstrated that the matrix metalloproteinase MMP-7 co-localizes with and cleaves the pro segment from intestinal alpha-defensin precursors. In humans, MMP-7 is expressed in a variety of glandular epithelia throughout the human body, making it an ideal candidate for the enzyme that processes hBD-1 and -2. In addition, preliminary data showed that MMP-7, like hBD-2, is prominently upregulated in epithelial cells exposed to bacteria, further supporting the idea that MMP-7 participates in host defense. This proposal will test the hypothesis that MMP-7 and the hBDs are co-regulated; furthermore, that MMP-7 mediates processing of beta-defensin precursors in airway tissue, thus producing more efficacious defense molecules at the mucosal surface. To test this hypothesis, the following specific aims are proposed: (1) assess co-expression of MMP-7 and beta-defensins produced by conducting airway epithelial cells both constitutively and following bacterial infection. This will address the likelihood that these molecules could be functionally interactive; (2) analyze the beta-defensin isoforms produced from airway cells and tissue in the presence of MMP-7. Determine if MMP-7 is required for beta-defensin processing in an in vivo-like context. This will be accomplished by using metalloproteinase inhibitors as well as genetically defined cells; and (3) quantitatively assess the antimicrobial properties of beta-defensin isoforms produced by MMP-7 cleavage. The proposed experiments should provide insight into the role of MMP-7 in mucosal epithelium and how this metalloenzyme contributes to mechanisms of host defense.

Grant: 1R21DE014160-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: CUTLER, CHRISTOPHER W PHD IMMUNOLOGY /
MICROBIOLOGY
Title: DENDRITIC CELLS, LPS AND ORAL MUCOSA
Institution: STATE UNIVERSITY NEW YORK STONY BROOK STONY BROOK, NY
BROOK
Project Period: 2000/09/15-2002/08/31

DESCRIPTION (Adapted from applicant's abstract): The oral cavity is a "portal of entry" for bacteria, fungi and other infectious agents that reach the respiratory and gastrointestinal tracts. Lining these tracts is the MALT, which can induce immunity or oral tolerance against bacteria species. The antigen-presenting cells known as DCs form a discrete microanatomical organization with T cells in MALT Peyer's patch, which is crucial for initiation and regulation of the immune response. Work from our laboratory and others have established the human oral mucosa as a rich repository of DCs. We presently understand little about the function and organization of DCs in these tissues. This R21 application proposes the hypothesis that, if proven, will support a new paradigm - that adaptive immunity is regulated at several levels by: (1) distinct DCs subsets that infiltrate the oral mucosa; (2) TLRs expressed; and (3) the antigens encountered. Our overall hypothesis is that the human oral mucosal DCs play pivotal roles in regulation of the adaptive immune response to specific antigens. The sub-hypotheses are: (1) distinct DC subsets infiltrate different microanatomical sites in the gingival/buccal mucosa; (2) these DC subsets are differentially responsive to LPS and other antigens by virtue of the expression of antigen-selective TLR2 and TLR4; and (3) the T cell response to antigens is regulated by DC responsiveness. The present application proposes the following aims: Aim 1: To characterize the DC subsets that infiltrate human oral mucosa in health and disease; and Aim 2: To determine the ability of DC subsets to regulate the T cell response to antigens in vitro. The PI has assembled a team of investigators with expertise in microbiology, oral pathology/stomatology, immunohistochemistry, LPS purification/characterization, DC/Langerhans cell function and T cell function to carry out these studies. The investigators are all located on the Dallas campus of the Baylor Medical Center Hospital, including Baylor College of Dentistry-TAMUS and the Baylor Institute for Immunology Research. It is hoped this research will advance our understanding of how adaptive immunity to microbial antigens is initiated and regulated at the mucosal level. Our short-term objective is to acquire sufficient preliminary data/publications to facilitate the successful submission of an R01 proposal(s). Our long-term objective is to develop this research to the level of a program project on DCs and the mucosal immune response.

Grant: 1U19DE013102-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: SCHENKEIN, HARVEY A PHD CLINICAL
DENT:PERIODONTIA
Title: GENETIC AND IMMUNOLOGIC ASPECTS OF PERIODONTAL DISEASES
Institution: VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA
Project Period: 2000/07/01-2005/06/30

Studies of genetic and immunologic aspects of periodontal diseases. The overall goal of the proposed research is to address significant questions in periodontology via an integrated approach that exploits investigator expertise in human genetics, immunology, and bacterial pathogenesis. The investigators participating in these projects have worked together successfully for many years and were most recently supported by a Periodontal Disease Research Center grant from NIDR. We now propose a series of projects tied together as a Program Project; we propose to support a Core facility that provides administrative, clinical, and biostatistical support to the other projects. Each project will rely upon the Core for support of its scientific goals. Four interrelated and integrated scientific projects are proposed. We have been studying genetic aspects of early-onset periodontal diseases (EOP) for several years and have identified a specific gene (IL-1 on chromosome 2q) and a chromosomal region (19q) involved in EOP susceptibility. Additional chromosomal regions are strongly suggested, providing a rationale for continuing and expanding these studies. Secondly, we have been studying antibody responses and immunoglobulin production in periodontitis patients; major findings have been the observations that serum IgG2 levels are a heritable trait, and that IgG2 serum concentrations are significantly higher in some EOP patients than in healthy individuals or AP patients. These observations form the foundation for 3 higher in some EOP patients than in healthy individuals or AP patients. These observations form the foundation for 3 additional projects. The contribution of cytokines, including Il-1 (which is involved in EOP susceptibility, and levels of which are genetically determined), and lipid mediators (including PGE2 and PAF) to IgG2 production will be examined. Further, the contribution of PAG regulatory enzyme PAG acetyl-hydrolase, which is produced in low concentrations by monocytes from LJP patients, will be studied. Additionally, we have discovered that IgG2 antibody levels against phosphorylcholine (PC) are elevated in patients with periodontal attachment loss compared to healthy subjects; this led us to the observations that anti-PC inhibits IgG2 production and that several plaque microorganisms associated with periodontal destruction (*A. actinomycetemcomitans*, *F. nucleatum*) and endocarditis (*S. sanguis*), may have PC. The significance of this a-PC antibody and oral bacterial PC will be examined, and genetic control of a-PC levels will be explored.

Grant: 2P01DK033506-16A1
Program Director: HAMILTON, FRANK A.
Principal Investigator: WALKER, W A MD CLINICAL MEDICAL
SCIENCES, OTHER
Title: BARRIER FUNCTION OF THE GI TRACT IN HEALTH AND DISEASE
Institution: MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA
Project Period: 1984/04/01-2005/09/29

In this Program Project renewal reapplication, we have continued a narrowed focus to characterize the role of the enterocyte in mucosal barrier function at the interface between microbial luminal stimuli and lymphoid effector response. The enterocyte is the central focus and will be studied with regard to microbial "crosstalk," lymphoid-epithelial interactions, inappropriate developmental responses, and as a barrier to microbial penetration. The renewal application consists of five interactive projects supported by two cores, (1) an Administrative Core and (2) a Tissue Culture/Morphology/Xenograph Transplant Core principally in one location in the Mucosal Immunology/Developmental Gastroenterology Laboratories at the Massachusetts General Hospital- East. Project 1 is a new project to study the enterocyte response, in immature human fetal cells, to exo- and endotoxin. Immaturities in signal transduction responses may account for the increased incidence of age- related inflammatory disease and secretory diarrhea in human infants. Project 2 will determine the inflammatory role of mast cell cytokines and leukotrienes in response to bacterial/enterocyte interaction. Project 3 will study the mechanisms of Shigella interaction with the basolateral surface of the enterocyte interaction. Project 3 will study the mechanisms of Shigella interaction with the basolateral surface of the enterocyte at the cellular mechanisms of Shigella interaction with the basolateral surface of the enterocyte interaction. Project 3 will study the mechanisms of Shigella interaction with the basolateral surface of the enterocyte at the cellular/molecular level. Two new projects have been added to the renewal application. Project 4 will define the role of neuropeptides, specifically corticotrophin releasing factor (CRF) in the intestinal inflammatory response to Clostridial toxins and Project 5 will determine the role of tight junctions, particularly claudin cell/molecular biology, microbiology, immunology, and developmental biology will work in collaborative fashion to define microbial/epithelial responses in the context of inflammation and mucosal defenses. These studies should provide a better understanding of the pathogenesis of bacterial gastroenteritis and lead to improved ways of preventing infectious diseases of the gastrointestinal tract.

Grant: 2P01DK046763-09
Program Director: HAMILTON, FRANK A.
Principal Investigator: TARGAN, STEPHAN R MD MEDICINE
Title: IBD--GENETIC AND IMMUNOPATHOLOGIC MECHANISMS
Institution: CEDARS-SINAI MEDICAL CENTER LOS ANGELES, CA
Project Period: 1992/09/30-2005/09/29

Research progress over the last four years, made under the auspices of the Program Project and independently by other investigators, has both supported our hypothesis and added great detail. Work supported by this inflammation in animal models and human disease. This very productive Program Project grant, entitled, "IBD: Genetic and Immunopathologic Mechanisms" is designed to approach the subject of inflammatory bowel disease from numerous vantage-points. Project 1, "Quantitative Linkage of Crohn's Disease Antibodies" has made major contributions to the understanding of the genetic susceptibility of IBD. Project 2, "IBD: Disease Subgroup Stratification by Immune Response to Commensal Bacterial Antigens" has made major contributions to current knowledge of disease stratification, and with Project 3, "Immune Mechanisms of Pathogenesis and Protection for Crohn's Disease (CD) Candidate Microbial Antigens," contributed to further understanding of marker antibodies of marker antibodies and relationship to bacterial antigens and T cell responses. Project 3 has also defined a unique CD associated antigen and shown disease associated cytokine responses in CD-like mouse models. Project 4, "Specificity of Homing of Pathogenic T cells in Mouse Colitis," has made important observations of the interplay of effector and regulatory T cells in mucosal inflammation. Our efforts have led us to a revised hypothesis guiding the Program Project renewal application as follows: Several genetic abnormalities affect the balance of T cell responses to commensal bacterial antigens. Disease susceptibility arises from an unfavorable genetic profile resulting in an imbalance of antigen specific effector or regulatory cells. These disordered T cell responses are associated with marker serum antibodies with cognate antigen specificity and isotype, and distinct patterns of mucosal damage resulting in the clinical manifestations of ulcerative colitis (UC) and CD. An important medical implication of this imbalance by inhibition of specific cytokines, augmentation of regulatory T cell function, or altering the bacterial microenvironment. The proposed hypothesis will be tested through an integrated set of human and animal model investigations in a highly interactive Program Project. The synergism between research in animal models and in human disease will be accelerated by the interplay of the projects in this Program Project grant. Core B in this proposal will be central and critical for accomplishing the objectives of human and animal studies. If the goals delineated in this proposal are accomplished, we will have a much cleared understanding of the genetic factors and immunologic processes controlling animal and human mucosal inflammation, and the relationship of the mucosal T cell imbalance to marker antibodies and responses to specific bacterial antigens.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2P01DK049720-06A1
Program Director: NYBERG, LEROY M.
Principal Investigator: WARREN, JOHN W
Title: MOLECULAR AND CELLULAR PATHOGENESES OF URINARY INFECTION
Institution: UNIVERSITY OF MARYLAND BALT PROF SCHOOL BALTIMORE, MD
Project Period: 1995/05/01-2005/06/30

Urinary tract infections (UTI) are among the most common infections in humans which can be asymptomatic or cause cystitis, acute pyelonephritis and blood stream invasion. About 80% of UTIs in otherwise normal urinary tracts, the incidence of UTI is much higher and is caused by a wider spectrum of organisms; among these, *Proteus mirabilis* assumes importance not only because of its production of renal stones. Among patients with catheters or other foreign devices in the urinary tract, Candidate infections are becoming increasingly prominent. In this PO1, a group of accomplished and resource investigators has the following objectives: 1) to discover the mechanisms of pathogenesis of UTI caused by *E. coli*, *P. mirabilis*. Our strategy is to use the powerful tools of molecular biology and a well established mouse model of UTI to examine known virulence factors and, via new techniques including signature tagged mutagenesis and in vivo expression technology, heretofore unknown virulence factors. Similar resources will be used to develop candidate vaccines assembled from *P. mirabilis* subunits. This work will be done in the context of a strong history in infectious diseases, particularly molecular pathogenesis and vaccine development.

Grant: 1P01DK055510-01A1
Program Director: HAMILTON, FRANK A.
Principal Investigator: LACKNER, ANDREW A DVM VETERINARY
MEDICINE
Title: AIDS AND THE GASTROINTESTINAL TRACT
Institution: HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA
Project Period: 2000/09/30-2005/02/28

HIV-infected patients commonly have unintentional weight loss and low serum micronutrient levels that have been correlated with disease progression. However, the relationship among these nutritional parameters, direct effects of the virus on the intestinal immune system and intestinal dysfunction and the role of opportunistic infections in progressive weight loss are not clear. These issues are extremely difficult to address in persons infected with HIV because a large percentage of HIV/AIDS patients are already taking a variety of nutritional supplements in an uncontrolled manner and dietary intake varies greatly. Furthermore, the rapid evolution of anti-retroviral therapy complicates obtaining answers to these questions. To obviate these problems and also to control for additional variable such as time since inoculation and differences in viral inocula we propose to use the simian immunodeficiency virus (SIV)_infected macaque model of AIDS. The organization of the Program Project involves three projects and four cores. Project 1 will define the natural history of weight loss and micronutrient deficiency in SIV-infected macaques and conduct two interventions to try and alleviate wasting. Project 2 will examine direct effects of SIV infection on the intestinal immune system and intestinal function. Project 3 will determine the relationship between key gastrointestinal opportunists (*C. parvum*, *E. bienensei*, and *M. avium*) and weight loss. Thus this Program Project represents a multi-disciplinary approach by a group of interactive, experienced investigators to examine the interplay between 1) nutrition, 2) direct effects of SIV infection and 3) opportunistic infections of the gastrointestinal tract on the pathogenesis of wasting.

Grant: 1P01DK057756-01
Program Director: HAMILTON, FRANK A.
Principal Investigator: FIOCCHI, CLAUDIO MD INTERNAL
MED:GASTROENTEROLOG
Title: PEDIATRIC IBD--KEY TO EARLY PATHOGENIC EVENTS
Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH
Project Period: 2000/09/15-2005/09/14

The overall goal of the Program Project is to fill a major gap in current knowledge of IBD pathogenesis by studying children with Crohn's disease and ulcerative colitis, and the progression of early to late stages of disease in animal models of gastrointestinal inflammation. The proposal is the natural outcome of a vigorous and multi-disciplinary expertise in IBD pathophysiology, intestinal immunity, mucosal cytokines, animal model of colitis and gastritis, and immune-non immune cell interaction in intestinal inflammation. The Program Project will investigate will investigate mechanistic links of early to late events of gut inflammation by testing the specific hypotheses proposed in four Research Projects. Project 1: Early IBD in children results from loss of tolerance to antigens of the commensal flora, and its persistence contributes to the chronicity of IBD. Project 2: Inflammation in murine models of IBD is initiated by mucosal T-cell responses to enterobacterial antigens, and it is perpetuated during the late stage of disease by a pro-inflammatory response maintained by mucosal immune and non immune cells. Project 3: Chronic gastrointestinal inflammation results from an aberrant immune response against antigenic stimuli derived from the normal enteric flora, and this response is modulated by immunization and the presence or absence of Helicobacter organisms. Project 4: Specific changes in the composition of the extracellular matrix of mucosal basement membrane contributes crucially to early inflammation, while altered synthesis and modulation of interstitial extracellular matrix foster progression of inflammation from the early to the late stages of disease. These projects will be performed using state-of-the-art methodological strategies including evaluation of tolerance to normal enteric antigens in pediatric and adult IBD patients, immunoregulatory studies in gene-deficient mice with early and late models of intestinal inflammation, colonization by commensal and infectious bacteria in these models, DNA microarray systems for differential gene expression in intestinal inflammation, extracellular matrix gene and protein expression in human and murine models of IBD, and induction of intestinal fibrosis in normal and proteoglycan-deficient mice. The four Projects will be supported by three Service Cores: a Patient Core (A), an Animal Core (B) and an Administrative Core (C).

Grant: 2R01DK037908-11
Program Director: LAUGHLIN, MAREN R.
Principal Investigator: HASSELGREN, PER-OLOF J
Title: MUSCLE PROTEIN TURNOVER AND AMINO ACID UPTAKE IN SEPSIS
Institution: UNIVERSITY OF CINCINNATI CINCINNATI, OH
Project Period: 1987/01/01-2004/07/31

Previous studies suggest that sepsis-induced muscle catabolism reflects ubiquitin-proteasome-dependent degradation of myofibrillar proteins regulated by glucocorticoids. Because intact myofibrils are not degraded by the proteasome, it is possible that actin and myosin are dissociated from the myofibrils before they are ubiquitinated and degraded by the proteasome. We will test the hypotheses: 1) sepsis results in glucocorticoid-mediated calcium/calpain-dependent Z-band disintegration and release of myofilaments in skeletal muscle; 2) sepsis results in increased N-end rule pathway-dependent ubiquitination and breakdown of muscle proteins and upregulated expression and activity of the ubiquitin-conjugating enzyme E2/14k and ubiquitin ligase E3alpha; 3) sepsis-induced muscle cachexia can be inhibited by proteasome blocker in vivo; 4) muscle cachexia in patients with sepsis is associated with increased expression and activity of calpains, release of myofilaments and upregulated protein breakdown in the N-end rule pathway. A septic model in rats consisting of cecal ligation and puncture is used in the majority of experiments. Total and myofibrillar protein breakdown rates are measured in incubated muscles by determining net release of tyrosine and 3-methylhistidine respectively. Integrity of sarcomeric Z-bands is studied by electron microscopy. Gene and protein expression of calpain and calpastatin are determined by Northern and Western blot analysis, respectively. The role of calcium/calpain-dependent proteolysis is assessed by the effect of dantrolene and diltiazem on sepsis-induced morphologic and metabolic changes. The role of glucocorticoids in sepsis-induced changes in muscle calcium levels and release of myofilaments is determined by the glucocorticoid receptor antagonist RU38486. To test the role of the N-end rule pathway, expression and activity of E2/14k and E3alpha are determined and specific E3alpha inhibitors are used in a cell-free system. Similar determinations are performed in muscle from patients with sepsis. The proposal is novel because it suggests that muscle cachexia during sepsis is caused by two distinct mechanisms, i.e., calcium/calpain-dependent release of myofilaments from the sarcomere followed by ubiquitination of myofilaments in the N-end rule pathway and subsequent degradation of ubiquitinated filaments by the 26S proteasome. The hypothesis implies two levels at which sepsis-induced muscle cachexia may be prevented/treated, i.e., inhibition of myofilament release by treatment with a calcium antagonist and inhibition of ubiquitin/proteasome-dependent degradation of the released myofilaments by a proteasome blocker.

Grant: 2R01DK040249-11A1
Program Director: HAMILTON, FRANK A.
Principal Investigator: SARTOR, R. B MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC
Title: MECHANISMS OF COLITIS INDUCED BY DEFINED BACTERIAL FLORA
Institution: UNIVERSITY OF NORTH CAROLINA CHAPEL HILL, NC
HILL
Project Period: 1989/02/01-2005/09/14

The etiology of IBD is unknown, but both genetic and environmental factors are involved. In the current funding period of this grant experimentally-induced and genetically engineered rodent models have been used to provide convincing evidence that the normal endogenous enteric bacterial flora is essential to the development of chronic colitis, gastritis and arthritis in genetically susceptible rodents. Very importantly, the use of HLA-B27/beta2 transgenic (TG) rats has demonstrated that all components of the resident bacterial flora are not equal in their capacity to induce inflammation: some are aggressive (*B. vulgatus*), some are neutral (*E. coli*) and some are protective (*Lactobacillus* sp). *B. vulgatus* from several sources causes more aggressive colitis in B27 TG rats than *B. distasonis* isolated from normal rats. Chronic intestinal inflammation in these models is mediated by activated T lymphocytes which are induced by normal luminal bacteria. These 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 tested by the following specific aims: 1) determine mechanisms by which *B. vulgatus* selectively induces colitis in HLA-B27 TG rats; 2) identify the mechanisms of immunologically determined susceptibility to *B. vulgatus* and other resident enteric bacterial components in HLA-B27 TG rats versus nontransgenic littermates. A NIH-funded Core Center for Gastrointestinal Biology and Disease at UNC supports a barrier-intact gnotobiotic rodent facility, providing the investigators with a unique environment to selectively colonize germ-free rats with defined luminal bacterial species. These studies will generate novel insights into the pathogenesis of IBD and open new opportunities for novel therapeutic interventions to block induction of antigen-specific immune response to luminal bacteria.

Grant: 2R01DK044632-09
Program Director: HAMILTON, FRANK A.
Principal Investigator: OUELLETTE, ANDRE J PHD
Title: PEPTIDE EFFECTORS OF ENTERIC HOST DEFENSE
Institution: UNIVERSITY OF CALIFORNIA IRVINE IRVINE, CA
Project Period: 1993/05/01-2005/04/30

Abstract Text Not Available

Grant: 2R01DK050411-05A1
Program Director: MAY, MICHAEL K.
Principal Investigator: SMITH, ROBERT J
Title: NUTRITION, CYTOKINES, AND ANABOLIC SIGNALING MECHANISMS
Institution: JOSLIN DIABETES CENTER BOSTON, MA
Project Period: 1995/09/01-2000/12/31

Patients with severe illnesses resulting from such diverse causes as trauma, infection, burn injury, major surgery, and certain cancers frequently develop a catabolic response that contributes to disease morbidity and complications, delays recovery, and may affect final disease outcome. Intensive nutrition support has significant benefit in catabolic patients, but current methods of nutrition support often do not reverse the catabolic state. In these patients, resistance to the actions of anabolic hormones, including growth hormone, appears to be a significant factor contributing to the catabolic response. The long-term objectives of this project are to define the molecular mechanisms that lead to growth hormone resistance during severe illness and, ultimately, use this information to develop more effective strategies for managing critically ill patients. During the previous grant period, specific signaling defects in the growth hormone pathway in the liver were shown to develop in rats in an experimental catabolic state induced by injection of endotoxin. Subsequent studies demonstrated that growth hormone resistance correlated with a marked increase in mRNAs for several of the recently described negative-regulatory SOCS (suppressor of cytokine signaling) genes. This has led to the hypothesis that cytokines elaborated in response to endotoxin activate SOCS genes as part of a negative feedback loop and, through a specificity- spillover mechanism, the SOCS proteins then inhibit growth hormone signaling. Additional studies have shown that SOCS mRNAs are increased by fasting or protein malnutrition, consistent with a role for these proteins in malnutrition-induced growth hormone resistance. The specific goals of this project are: (1) to define the extent and mechanisms of nutritional regulation of SOCS genes in rat liver and skeletal muscle, (2) to investigate the functional effects of SOCS proteins on GH signaling in liver tissue in vivo using recombinant adenovirus-mediated gene transfer, (3) to investigate the synergistic effects of malnutrition and endotoxin in the induction of SOCS genes, and (4) to examine the effects of the tyrosine phosphorylation modifying agent vanadyl sulfate as a potential pharmacological approach to decreasing growth hormone resistance. The work will investigate fundamental molecular mechanisms of the catabolic response in pathophysiologically relevant experimental models with the ultimate objective of developing new approaches to the use of nutritional, hormonal, and pharmacological interventions in the treatment of catabolic patients.

Grant: 1R01DK054777-01A2
Program Director: MAY, MICHAEL K.
Principal Investigator: BENYA, RICHARD V MD
Title: ROLE OF GALANIN IN INTESTINAL PATHOPHYSIOLOGY
Institution: UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL
Project Period: 2000/06/15-2000/12/15

Abstract Text Not Available

Grant: 1R01DK055496-01A1
Program Director: EGGERS, PAUL
Principal Investigator: MARRS, CARL F
Title: MOLECULAR EPIDEMIOLOGIC APPROACHES TO UTI GENE DISCOVERY
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2000/03/17-2005/02/28

DESCRIPTION: (Adapted from Investigator's Abstract) In 1995, 11.3 million women in the United States had urinary tract infection (UTI) with associated costs of approximately \$1.5 billion. *Escherichia coli* accounts for about 90 percent of all UTI present in ambulatory patients. Several different pathogenic processes probably underlie *E. coli* UTI, analogous to the several different mechanisms that explain diarrheas caused by *E. coli*. A limited number of *E. coli* UTI virulence genes have been identified. Known genes in *E. coli* UTI were identified primarily by phenotypic expression of traits (hemagglutination, toxic activity on tissue culture cells, etc.) hypothesized to be important for colonization or virulence, and then tested by epidemiologic correlations or animal model studies. The goal of this study is to identify additional genes involved in *E. coli* UTI virulence, transmission, or duration of colonization. The investigators propose the use of a novel approach involving a four stage process, as follows: 1) epidemiologic pairing of bacterial strains with the highest potential to identify important new genes; 2) differential cloning of DNA regions present in one member of the pair but not in the other through genomic subtraction; 3) epidemiologic screening of the resulting DNA regions for association with UTI transmission, virulence, and maintenance using the investigators' well characterized collections of UTI and fecal *E. coli* isolates; and 4) characterization of potential UTI genes in the associated DNA regions. The investigators state that the discovery of additional factors involved in *E. coli* mediated UTI will facilitate the development of new strategies for the prevention and cure of UTI.

Grant: 1R01DK056754-01A1
Program Director: HAMILTON, FRANK A.
Principal Investigator: MC CORMICK, BETH A PHD
Title: INTESTINAL INFLAMMATION ORCHESTRATED BY PATHOGENS
Institution: MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA
Project Period: 2000/09/15-2005/08/31

The active phase of both Salmonella-associated gastroenteritis and chronic states of inflammatory bowel disease (IBD), such as ulcerative colitis and Crohn's disease, is characterized histologically by polymorphonuclear leukocyte (PMN) migration into and across the epithelial lining of the intestine. These events result in acute inflammation of the epithelium and subsequent epithelial dysfunction. The degree of PMN transmigration into intestinal crypts and the formation of crypt abscesses is indicative of disease severity and is used clinically to evaluate the activity of IBD. It is unclear what triggers directional movement of PMN across the intestinal epithelium. Towards this end, we have recently shown that epithelial cells themselves can send such signals to underlying PMN and these signals are regulated by enteric flora, such as *S. typhimurium*. The broad long term objectives of this proposal are to investigate the molecular mechanism by which epithelial cells in response to microbial pathogens can signal to PMN and orchestrate their directed migration. Once we begin to understand the basis of such transcellular signaling important in promoting disease flares of *S. typhimurium* pathogenesis, it may be possible to develop novel therapeutic strategies aimed at treatments for and ameliorating IBD. The specific aims are ultimately directed at achieving this goal, and are three-fold. Specific Aim 1 is designed to determine the nature of *S. typhimurium* virulence factors and define their contribution to the epithelial orchestration of mucosal inflammation. Specifically, we will delineate how *S. typhimurium* SipA, SopB, and SopA secreted proteins interfere with the signaling pathways which lead to epithelial orchestration of mucosal inflammation by expression of these proteins in epithelial cells using adenoviral expression vectors. Functional effect of expression of these proteins on orchestration of proinflammatory events which govern PMN transepithelial migration will be correlated with morphological consequences by both confocal and electron microscopy. Specific Aim 2 is designed to identify the signal transduction cascades which lead to the release of the proinflammatory chemoattractant PEEC and will employ several different approaches which include determining the relationship between *S. typhimurium* invasion and the apical epithelial release of PEEC, examination of the role of the JNK-pathway, determining the effects of small GTPase (*cdc42*, *rac-1*, and *Arf6*) expression on the ability of *S. Typhimurium* to induce PMN transepithelial migration by expression of dominant inhibitory mutants using adenoviral expression vectors, examining the role of phosphoinositide signaling, and determining whether the ability of *S. typhimurium* to elicit PEEC secretion correlates with their ability to induce an increase in intracellular calcium in model intestinal epithelia. Specific Aim 3 is designed to characterize a recently identified pro-inflammatory PMN chemoattractant. The first part of this aim will elucidate the structure of PEEC utilizing HPLC purification, NMR analysis, mass spectrometry and sequence analysis, while the second part of this aim will define PEEC's relationship to other PMN chemoattractants including its ranking in the PMN chemoattractant hierarchy, will determine whether PEEC is able to activate other immune-type cells as well as assess the role of PEEC in inflammation.

Includes Research Project Grants (RPGs)

Excludes clinical trials

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01DK056903-01A1
Program Director: NYBERG, LEROY M.
Principal Investigator: WU, XUE-RU MD
Title: ROLE OF TAMM-HORSFALL PROTEIN IN URINARY TRACT DEFENSE
Institution: NEW YORK UNIVERSITY SCHOOL OF MEDICINE NEW YORK, NY
Project Period: 2000/09/15-2005/06/30

DESCRIPTION (Adapted from the Applicant's Abstract): Urinary tract infection (UTI) is a major clinical problem caused primarily by *E. coli*. Most uropathogenic *E. coli* strains express type 1 fimbriae, an important urovirulence factor that mediate *E. coli* adhesion to specific urothelial receptors. Recent data from the investigators' laboratory and others showed that uroplakins Ia and Ib, two major protein subunits for urothelial plaques that cover >80% of the urothelial surface, are the major urothelial receptors of the type 1-fimbriated *E. coli*. It has been suggested, however, that the binding of the bacteria to urothelial receptors can be blocked by soluble receptors present in the host urine. One soluble receptor candidate is Tamm-Horsfall protein (THP), the most abundant protein in human urine. Their preliminary data indicate that THP can bind via its high-mannose moiety to type 1-fimbriated *E. coli*, and block the binding of type 1-fimbriated *E. coli* to urothelial receptors in vitro. These results strongly suggest a possible defensive role of THP against *E. coli* infection. The main goal of this study is to evaluate the in vivo function of THP, first focusing on its role in host urinary defense against type 1-fimbriated *E. coli* infection. Toward this end, they will perform three series of experiments: (1) ablate the THP gene by conventional in vivo knockout in which THP gene will be ablated during embryonic development, as well as by inducible knockout in which THP gene will be ablated in adult mice, and examine the susceptibility of transgenic mice to *E. coli*-induced UTIs; (2) map and characterize the *E. coli*-binding, high mannose-type glycosylation site(s) of THP isolated from human and mouse urine, and recombinant THP expressed in cultured epithelial cells; and (3) selectively mutate the high-mannose glycosylation site(s) of THP in transgenic mice and test the effects of the absence of this unique glycosylation of THP on urinary defense. These studies should lead to a better understanding of the physiological function of THP, the molecular pathogenesis of urinary tract infections, and the host defense mechanisms of the urinary tract.

Grant: 1R01DK057827-01
Program Director: MAY, MICHAEL K.
Principal Investigator: MADARA, JAMES L MD OTHER AREAS
Title: CRYPTDIN EFFECTS ON CRYPT EPITHELIA
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 2000/06/01-2005/05/31

DESCRIPTION (applicant's abstract): The goal of this proposal is to elucidate the biology of cryptdin-induced channel formation in crypt epithelia. Cryptdins are intestinal (Paneth cell) specific alpha-defensins - cationic peptides first recognized as neutrophil products, which exhibit potent antimicrobial properties. Nineteen cryptdin isoforms have been described in the mouse and two, HD-5 and HD-6, in the human. The antimicrobial action of alpha-defensins is thought to reside in their ability to partition into biomembranes and produce anion conductive pores. Cryptdins, and HD-5 and HD-6 are known to be released from Paneth cells into the crypt lumen and thus other crypt cell types, the major one being the chloride secreting "undifferentiated" crypt cell, are naturally exposed to these channel forming peptides. The PI and his collaborators have recently shown the cryptdins 2 and 3 induce a physiologic chloride secretory response in human intestinal T84 cells likely by forming anion conductive channels in the apical (luminal) membrane. Studies proposed will test the hypothesis that Paneth cell-derived cryptdins act to regulate the physiology of the intestinal crypt by paracrine insertion (self-assembly) of chloride conducting pores into apical membranes of neighboring crypt epithelial cells and down regulation by endocytosis and apical membrane restitution. In vitro experiments will define the mechanism by which mouse cryptdins 2 and 3 induce chloride secretion. The PI and his collaborators will use matrilysin $-/-$ mouse and CR2-tox176 mouse to elucidate the effects of cryptdins on small intestinal functions ex vivo and in vivo. The biophysical properties and structure function of cryptdin 3 and HD-5 and HD-6 will be examined in intact and semi-permeabilized T84 and renal HEK cell model systems and by single channel patch clamp. Finally studies are proposed to elucidate the cellular mechanism(s) of cryptdin 3-induced channel formation and down regulation. The significance of these studies is emphasized by accumulating evidence that the intestinal crypt plays a central role in intestinal epithelial cell ontogeny, physiology (solute and water transport), and specific and non-specific mucosal defense. Cryptdins act in paracrine fashion to regulate crypt cell function and appear to have a unique mechanism of action. Proposed studies will elucidate the biology of these novel alpha-defensins and may set the stage for future development of alpha-defensin inhibitors or for use of these (or related peptides) in treatment of disease at the mucosal surfaces of the intestine or respiratory tract.

Grant: 1R01DK057879-01
Program Director: HAMILTON, FRANK A.
Principal Investigator: WALKER, W. A MD CLINICAL MEDICAL
SCIENCES, OTHER
Title: BACTERIAL TOXIN ACTION ON THE DEVELOPING HUMAN GUT
Institution: MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA
Project Period: 2000/07/01-2000/10/01

Diarrheal disease constitutes one of the major causes of morbidity and mortality in infants and children on a global scale. We know from clinical studies that an inappropriate initial bacterial colonization of the premature intestine may result in severe inflammation leading to an intestinal disease unique to the premature, e.g.- necrotizing enterocolitis (NEC) and that certain toxigenic diarrheas occur more commonly and are manifested more severely in the neonatal period. In preliminary studies in human intestinal models (cells lines, organ culture, Ussing chambers, and xenotransplants), we provide data that the immature human intestine inappropriately responds to bacterial toxin by secreting excessive IL-8, a chemokine for neutrophils, (endotoxin) and by excessive chloride secretion (exotoxin) Based on these observations, our overall hypothesis for this research proposal is that the pathogenesis of neonatal bacterial inflammatory intestinal diseases and certain secretory diarrheas involving bacterial toxins is principally due to an immature (inappropriate) enterocyte response to the bacterial toxin stimulation. In order to test this hypothesis, we will use two toxin-enterocyte "crosstalk" paradigms in human intestinal models to characterize the epithelial response and the mechanisms of this response in the immature compared to the mature intestine. Accordingly, our specific aim are: (1) to examine endotoxin interaction with the fetal enterocyte using IL-8 secretion as the effector response by examining LPS-LBP-CD14 interaction with toll-like receptors (TLRs) and postreceptor signal transduction events (principally the IL-1 signal transduction pathway leading to NFkappaB activation) and (2) to study exotoxin-fetal enterocyte interaction using Cl- secretion as the effector response by examining toxin binding and postreceptor responses via cAMP, GSalph, ribosylation factors, effector expression and phosphorylation and other pathways mediated by PGE2 and 5-HT. Having examined each step in the interaction of endo- and exotoxin with the developing intestine, we will attempt to modulate any identifiable step that is developmentally regulated by using known maturational (trophic) factors using the developmentally regulated step itself as the effector response. These studies may provide the basis for using a specific trophic factor or combination of trophic factors in the prevention of toxigenic diarrhea in premature and neonatal infants.

Grant: 1R01DK058536-01
Program Director: MAY, MICHAEL K.
Principal Investigator: CASANOVA, JAMES E
Title: MECHANISMS OF SALMONELLA INVASION AND TRANSMIGRATION
Institution: UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA
CHARLOTTESVILLE
Project Period: 2000/03/01-2004/02/28

Salmonella pathogenesis is characterized by the invasion and penetration of the intestinal epithelial barrier. Entry into host cells requires dramatic reorganization of the actin cytoskeleton, which in non-polarized cells is mediated by members of the Rho family of GTPases. However, the apical actin cytoskeleton of epithelial cells is highly specialized, and we have found that a member of the ADP-ribosylation factor (ARF) family of GTPases, ARF6, may also mediate Salmonella internalization in polarized epithelial cells. In Specific Aim 1, we will examine the specific roles of Rho GTPases and ARF6 in Salmonella internalization and signaling at the apical plasma membrane. Once inside the host cell, Salmonella reside within vacuolar bodies derived from host cell membranes that support their intracellular replication, transit to the basolateral plasma membrane and subsequent release into the lamina propria. These vacuoles are essentially large and specialized endosomes diverted by the bacteria from normal membrane trafficking pathways, and their composition changes with time as the bacteria migrate from the apical to basolateral pole of the cell. Using an MDCK cell model, in which epithelial cells are cultured on permeable filter supports, we have shown that the emergence of bacteria from the basolateral pole of the cell is a vectorial, non-random process, suggesting that Salmonella recruit components of the host cell targeting machinery to direct their transport to and fusion with the basolateral membrane. In support of this hypothesis, we have identified one member of the rab family of small GTPases, rab5, that is present on Salmonella-containing vacuoles at an early stage of biogenesis. In Specific Aim 2, we will determine the function of rab5 in vacuolar maturation, and identify other rabs that may participate in this process. It is widely accepted that IgA protects mucosal surfaces from bacterial invasion by preventing the adhesion of bacteria and their products to the epithelial cell surface. However, it has also been hypothesized that IgA may act intracellularly, to inhibit the replication of and enhance the clearance of intracellular pathogens from the cell. In Specific Aim 3, we will determine whether intracellular IgA interacts with transmigrating Salmonella, and whether such interaction is sufficient to perturb vacuolar biogenesis or transport. The results of these studies will provide significant insight into the mechanisms of Salmonella pathogenesis within the intestinal epithelium.

Grant: 1R01DK058953-01
Program Director: HIRSCHMAN, GLADYS H.
Principal Investigator: KOHAN, DONALD E MD OTHER AREAS
Title: EFFECT OF SHIGATOXIN-1 ON BRAIN ENDOTHELIAL CELLS
Institution: UNIVERSITY OF UTAH SALT LAKE CITY, UT
Project Period: 2000/09/30-2005/07/31

DESCRIPTION (adapted from the application) HUS is the leading cause of acute renal failure in children and is characterized by renal injury, microangiopathic hemolytic anemia, and thrombocytopenia. Although the kidney is an initial target in HUS, those patients who die from the disease do so primarily from brain, not renal, involvement. There is, however, very little understanding of how the central nervous system is affected in this disorder. HUS is associated with enteric infection by Shiga toxin (Stx) producing E. coli. The toxin binds to cells expressing a specific glycosphingolipid cell surface Stx receptor (Gb3) whereupon it may exert a variety of effects, including inhibition of protein synthesis, induction of apoptosis, regulation of vasoactive factor production, and others. Generally, these studies have focused on cells thought to be primary targets in HUS, namely, renal cells. Very little work, however, has been done on how Stx affects the brain. Preliminary studies from our laboratory indicate that human brain microvascular endothelial cells (HBEC) might be targets of Stx action. Further, these studies suggest that HBEC may respond to Stx and factors likely to be present in the setting of HUS in highly unique manner. Based on these findings, the following hypothesis has been formulated: Unlike renal endothelial cells, HBEC are not normally sensitive to Stx. Soluble or cell-associated members of the inflammatory cytokine superfamily, derived from circulating white blood cells or endothelial cells themselves, cause massive upregulation of Stx responsiveness in HBEC. Such upregulation leads to enhanced white blood cell and possibly platelet adhesion, endothelial cell apoptosis and necrosis, and altered vasoactive factor production. The unique responsiveness of HBEC to cytokine upregulation of Stx-1 sensitivity may provide the basis for therapeutic interventions aimed at blocking cytokine actions on the brain. Accordingly, the specific aims are: 1) Determination of HBEC sensitivity to the cytotoxic and protein synthesis inhibitory effects of Stx-1; 2) Determination of inflammatory factor regulation of the cytotoxic effect of Stx-I in HBEC; 3) Determination of the source(s) of inflammatory cytokines that affect HBEC responsiveness to Stx-1, focusing on HBEC and circulating white blood cells; and 4) Determination of the effects of Stx-1 on HBEC that could lead to CNS dysfunction in HUS, including mechanisms of cytotoxicity, regulation of vasoactive factor production, and modulation of platelet adherence.

Grant: 1R01DK058957-01
Program Director: HAMILTON, FRANK A.
Principal Investigator: KAPER, JAMES B PHD
MICROBIOLOGY: BACTERIOLOGY
Title: NOVEL E. COLI O157:H7 INTESTINAL COLONIZATION FACTORS
Institution: UNIVERSITY OF MARYLAND BALT PROF SCHOOL BALTIMORE, MD
Project Period: 2000/09/30-2005/07/31

DESCRIPTION (adapted from the application) The essential first stage of typical E. coli O157:H7 infection is colonization of the intestinal tract. To date, only one bacterial factor has been shown to mediate intestinal adherence by O157:H7, the outer membrane protein intimin which was originally discovered in the Principal Investigator's laboratory. However, a variety of observations indicate that additional intestinal colonization factors may play a role in disease due to E. coli O157:H7 and Shiga toxin-producing E. coli of serotypes other than O157:H7. The overall goal of this project is to investigate the mechanisms by which E. coli O157:H7 and non-O157:H7 enterohemorrhagic E. coli colonize the intestine. Our approach will be focused in four specific aims. Three of the specific aims will focus on characterization of three newly discovered potential colonization factors of EHEC. Two of these factors have homology to previously described adhesins in other enteric pathogens and the third factor has no obvious homology to previously described adhesins. For each of these factors we will construct isogenic non-polar mutations in the structural gene and test the mutants for decreased adherence in differential intestinal epithelial cell lines, freshly harvested human intestinal epithelial cells, and in a gnotobiotic piglet model of disease. The demonstrated involvement of any of the three novel colonization factors in intestinal adherence would provide new targets for vaccine development. The fourth aim will focus on a novel regulatory mechanism of the best-characterized EHEC colonization factor, intimin. We recently showed that in vitro expression of the intimin adhesin and the type III secretion system encoded on the LEE pathogenicity island is positively regulated by autoinducer molecules expressed by normal flora E. coli strains, i.e., quorum sensing. We propose to test whether quorum sensing is active in vivo by constructing an O157:H7 derivative that no longer responds to quorum sensing and testing it for colonization in a pig model. If quorum sensing is active in vivo, this would open up a new area for potential therapeutic intervention based on inhibiting the quorum sensing mechanism, thereby decreasing expression of intestinal colonization factors.

Grant: 1R01DK058960-01
Program Director: HAMILTON, FRANK A.
Principal Investigator: KAGNOFF, MARTIN F
Title: INTESTINAL EPITHELIAL RESPONSES TO FOOD BORNE PATHOGENS
Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA
Project Period: 2000/09/30-2005/07/31

A single layer of epithelial cells separates the intestinal lumen from the host's internal milieu. Clinically relevant foodborne pathogens may reside in the intestinal lumen in close contact with epithelial cells, adapt an intraepithelial cell lifestyle, or invade the epithelium as well as deeper layers of the intestinal mucosa and spread systemically. Despite these markedly different microbial lifestyles, and various mechanisms for causing host disease, a common feature of foodborne pathogens is their interaction with the host intestinal epithelium. Therefore understanding host intestinal epithelial cell responses to foodborne pathogens is central to the development of strategies to alter the host-pathogen interaction to the advantage of the host. The overall long-term goal of our studies is to define the repertoire and functional relevance of intestinal epithelial cell responses to foodborne pathogens. We hypothesize that intestinal epithelial cells express a relatively narrow repertoire of responses that are relevant to host innate and acquired immune defense following infection with foodborne pathogens. The proposed studies focus on three types of intestinal epithelial responses to foodborne pathogens that we consider will be particularly important for host innate and acquired defense. The three specific aims of the proposal are 1) to characterize the regulated expression and possible autocrine/paracrine function of intestinal epithelial cell expressed MIP-3alpha in the host response to foodborne pathogens; 2) To define epithelial cell apoptosis as an important host response to infection with foodborne pathogens; and 3) To define the regulated expression of antimicrobial peptides by human intestinal epithelial cells in response to epithelial cell infection with foodborne pathogens. These studies will use Salmonella as a model of an enteroinvasive foodborne pathogen, Cryptosporidium parvum as a model intraepithelial pathogen, and Escherichia coli O157:H7 as an intraluminal pathogen that lives in intimate association with the apical intestinal epithelial cell membrane. Our experimental approaches will draw on in vitro and in vivo model systems established in this laboratory and a duality of hypothesis and discovery based experimental approaches.

Grant: 1R01DK058964-01
Program Director: HAMILTON, FRANK A.
Principal Investigator: HECHT, GAIL A MD
MEDICINE/GASTROENTEROLOGY
Title: ENTEROHEMORRHAGIC E COLI ALTER INTESTINAL FUNCTION
Institution: UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL
Project Period: 2000/09/30-2005/07/31

Infection with enterohemorrhagic *E. coli* (EHEC), acquired from contaminated food or water, represents a major health problem worldwide with EHEC being the fourth most costly foodborne pathogen in the USA. Although many EHEC strains produce Shiga toxin (Stx), its role in pathogenesis is not understood. In some species, the presence of attaching and effacing (A/E) lesions correlate more closely with intestinal symptoms than does the expression of Stx. While the formation of A/E lesions may be sufficient to induce diarrhea, the production of Stx may be a prerequisite for hemorrhagic colitis. These findings underscore the lack of understanding of the pathogenesis of EHEC. While much effort has been focused on the extraintestinal manifestations of EHEC, the effects of this important pathogen on the intestine have been grossly understudied. The hypothesis of this proposal is that EHEC has direct effects on its initial host target tissue, the intestinal epithelium, which contribute to the associated symptoms. The studies proposed here will utilize two separate models to investigate the effects of EHEC infection on intestinal epithelial function, a reductionist model of cultured human intestinal epithelia and a murine model. Preliminary data show that two major physiological processes, tight junction (TJ) barrier function and the epithelial-initiated inflammatory cascade, are altered in both of these models following infection with EHEC. It is likely that both of these alterations contribute to EHEC-associated diarrhea. The impact of EHEC infection on these physiological functions and the underlying mechanisms will be explored by three Specific Aims. The first Specific Aim is to characterize the effect of EHEC on intestinal epithelial tight junction barrier function. The mechanisms by which EHEC perturbs the TJ barrier will be addressed focusing on the role of cytoskeletal contraction and changes in key TJ-associated proteins. Specific Aim 2 is to define the signaling pathways by which EHEC activates the inflammatory response within intestinal epithelial cells. The events that lead to the activation of NF-kappaB, the upregulation of pro-inflammatory cytokines, such as IL-8, and neutrophil transmigration will be explored. The studies outlined in Specific Aims 1 and 2 will use the established in vitro cell culture model. Specific Aim 3 is to establish and characterize a murine model of intestinal EHEC infection, The effects of EHEC infection on TJ barrier function as well as epithelial-initiated inflammation will be explored.

Grant: 1R01DK058993-01
Program Director: HIRSCHMAN, GLADYS H.
Principal Investigator: TZIPORI, SAUL V DVM VETERINARY
MEDICINE
Title: RECOMBINANT SHIGA-TOXIN-SPECIFIC HUMAN ANTIBODIES
Institution: TUFTS UNIVERSITY BOSTON BOSTON, MA
Project Period: 2000/09/30-2005/07/31

This proposal is in response to RFA: DK-00-005 "Foodborne Illness, Gastrointestinal and Renal Complications", specifically addressing "Development of interventions and/or clinical strategies to prevent complications of E. coli 0157:H7-related illnesses during the initial critical interval between the occurrence of hemorrhagic colitis and the development of HUS". The goal of this proposal is to develop an effective immune-based formulation which can be administered safely to children at risk of developing Shiga-toxin (Stx)-related hemolytic uremic syndrome (HUS). The target populations for this treatment include children in whom the disease can directly or indirectly be attributed to Stx. Currently there is no effective treatment or prevention for HUS. In this proposal we demonstrate that exogenous Stx-specific human monoclonal antibodies (Hu-mAbs) which we have generated under a separate NIH award, protect gnotobiotic piglets against Stx-mediated fatal neurological symptoms when administered 6-12 hours after oral challenge with E. coli 0157:H7. In contrast, piglets treated with placebo develop vascular-mediated fatal neurological symptoms within 2-3 days after the oral challenge. In this application we wish to express several of these anti-Stx Hu-mAbs in an eukaryotic system. This will allow us to produce them in large quantities (Specific Aim 1) and in different forms, such as isotype-variants (Specific Aim 2) and Fab fragments (aim 3) to determine which form would provide the most effective protection in vitro and in vivo (Specific Aim 4). We anticipate the final product will contain a cocktail of Hu-mAbs active against the A and B subunits of Stx1 and Stx2 (Specific Aim 4). Given our Preliminary Data, we are confident that specifically designed and highly concentrated Hu-mAbs will be safe and effective in protecting children at risk of HUS. We believe recombinant Hu-mAbs, either as whole molecules or Fabs, will be equally effective. Recombinant Hu-mAbs will considerably improve the efficiency of production of these reagents and make them available for clinical use. In contrast to other sources, such as polyclonal antibodies or murine/chimeric mAbs, Hu-mAbs have longer half-life, better affinity for targets, higher potency, require a lower dose, are safer, and are clearly the wave of the future. We are confident that at the end of the five-year support period we will have expressed in an eukaryotic system, a fully characterized panel of effective recombinant Stx-specific Hu-mAbs, ready to be produced under FDA guidelines for clinical evaluation.

Grant: 1R01DK059004-01
Program Director: HIRSCHMAN, GLADYS H.
Principal Investigator: GEAR, ADRIAN R DPH
Title: SHIGA TOXIN INDUCED INFLAMMATION AND THROMBOSIS
Institution: UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA
CHARLOTTESVILLE
Project Period: 2000/09/30-2005/07/31

DESCRIPTION (adapted from the application) The overall objective of the proposed research is to understand the thrombotic complications associated with Shiga-toxin infection. Platelet-rich thrombi in the kidney may contribute significantly to the clinical problems, but little is known about the factors responsible for the generation of these thrombi. Platelet function is not directly activated by Shiga toxin, indicating that other mechanisms must be involved. Shiga toxin binds to receptors on epithelial and endothelial cells leading to inhibition of protein synthesis and a stress which can result in local inflammatory responses. Monocytes and macrophages can also release inflammatory chemokines and certain ones are powerful co-activators of platelet function, such as SDF-1 (stromal cell-derived factor 1) and MDC (macrophage-derived chemokine). We will therefore test the hypothesis that chemokines contribute to the vascular and thrombotic problems of Shiga-toxin infection. Our proposed research has three main aims directed to the above hypothesis. First, endothelial (EC) and epithelial (EP) cells and then monocytes and macrophages will be exposed to Shiga toxin and the cell media tested for their ability to activate platelet function, as assessed by aggregation, adhesion and secretion. Second, we propose to identify the chemokines and other factors in the media which may be responsible for platelet stimulation. Then, we plan to analyze whether chemokine-treated platelets adhere to EC previously exposed to chemokines under varied shear-stress conditions. New quenched- and continuous-flow techniques developed in our laboratory will be employed to follow not just the rapid functional kinetics (less than 5 sec), but also platelet biochemical events. Third, we will examine the need for low levels of primary platelet agonists such as ADP, to enable Shiga-toxin effects on function, using new blocking reagents and treatment of platelets with apyrase. Finally, the role of proteases in the Shiga-toxin effects on platelet function will be examined.

Grant: 1R01DK059010-01
Program Director: HAMILTON, FRANK A.
Principal Investigator: BUTTERTON, JOAN R MD
Title: NEW ANIMAL MODEL FOR EHEC PATHOGENESIS AND PREVENTION
Institution: MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA
Project Period: 2000/09/30-2005/07/31

DESCRIPTION (adapted from the application) Investigation of the gastrointestinal disease and renal injury caused by enterohemorrhagic Escherichia coli (EHEC) and the development and testing of interventions to prevent disease following infection have been hampered by the lack of a convenient animal model that effectively reproduces the typical human colonic disease that progresses to HUS. The Principal Investigator proposes to develop the use of a new mouse model of EHEC infection with the long term goal of increasing the ability to study disease pathogenesis and prevention. The new animal model will be compared with prior models in the evaluation of vaccine strategies against EHEC. This will be accomplished through the following two Specific Aims: 1. Evaluation of the use of *Citrobacter rodentium* expressing Stx in a mouse model of Shiga toxin-producing E. Coli (STEC) infection. *C. rodentium*, a naturally occurring pathogen of laboratory mice which causes transmissible murine colonic hyperplasia, binds to the mouse enterocyte by a specific attachment and effacement lesion similar to that of EHEC. Strategies have been developed to lysogenize *C. rodentium* with antibiotic-marked Stx1- and Stx2- expressing bacteriophages as well as to express toxin components from plasmid vectors. Toxin production, phage induction, and lysogen stability will be evaluated in vitro. Mice will be challenged with toxin-producing *C. rodentium* and evaluated for clinical and pathologic signs of disease. This model has the potential of reproducing both the gastrointestinal and renal injury seen in EHEC infection, allows the use of adult animals and the development of normal immune responses, utilizes the power of mouse genetics to investigate genetic factors in determining gastrointestinal (GI) and systemic disease expression, and provides a significant increase in the ease of identifying and testing new interventions compared to many other animal models. 2. Expression of nontoxic Stx1 and Stx2 antigens using a balanced lethal plasmid system in *Vibrio cholerae* vaccine strains. *Vibrio cholerae* will be used as a live oral attenuated vaccine vector to deliver immunogenic antigens of EHEC to stimulate a common mucosal immune response. A balanced lethal plasmid system will be used to provide stable expression of the heterologous antigens from the vaccine strains. The germfree mouse model of *V. cholerae* colonization will be used to examine mucosal and systemic immune responses to the toxin components expressed by the vector strains. The new mouse challenge model will be compared to prior mouse models in evaluating protection from disease in response to immunization with *V. cholerae* strains expressing the EHEC antigens.

Grant: 1R01DK059811-01
Program Director: HIRSCHMAN, GLADYS H.
Principal Investigator: NEWBURG, DAVID S
Title: ENDOTHELIAL GB3 SPECIES INVOLVED IN HUS PATHOGENESIS
Institution: UNIV OF MASSACHUSETTS MED SCH WORCESTER, MA
WORCESTER
Project Period: 2000/09/30-2004/07/31

DESCRIPTION (adapted from the application) Acute renal failure and central nervous system (CNS) injury are life-threatening complications of hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (HUS/TTP). Survivors of HUS/TTP sometimes suffer irreversible damage. This application tests two hypotheses regarding the binding and toxicity of Shiga toxin (Stx) of enterohemorrhagic Escherichia coli (EHEC) to renal and cerebral endothelial cells, a critical process in HUS/TTP pathogenesis: (1) Metabolic regulation of globotriaosylceramide (Gb3) and galactosyl Gb3 (Gal Gb3), the glycolipid receptor(s) for Stx in endothelial cells, is central to HUS/TTP. (2) Binding to specific Gb3/Gal Gb3 molecular species is responsible for biological activity of Stx. Specific fatty acid moieties in the ceramide portions of Gb3/Gal Gb3 have been shown to influence Stx binding, and specific Gb3 species relate more strongly than total Gb3 to Stx-induced injury of endothelial cells from human saphenous veins. Using the cells most relevant to HUS/TTP, human glomerular endothelial cells (hGEC) and human cerebral endothelial cells (hCEC), investigations will (1) Isolate and measure individual Gb3/Gal Gb3 species in hGEC and hCEC cultures incubated with and without TNF-alpha and IL1-beta. (2) Determine which Gb3 and Gal Gb3 species relate to Stx1 and Stx2 toxicity in cytotoxin-stimulated and unstimulated cultures and to Stx binding to cells. (3) Define the metabolic pathways that synthesize the individual molecular species of Gb3/Gal Gb3 in response to TNF-alpha or IL1-beta stimulation by selectively inhibiting previously described pathways. Determine which UDPgalactose: lactosylceramide alpha1, 4 galactosyltransferase isozymes control this synthesis. Elucidation of the different sensitivities of renal and cerebral cells to Stx may explain differences in the pathogenesis of renal and CNS disease in HUS/TTP. The detailed description of the expression of these endothelial cell Stx receptors will potentially lead to development of precisely targeted therapies that can reduce Stx toxicity and these devastating effects of HUS/TTP.

Grant: 1R03DK056938-01
Program Director: PODSKALNY, JUDITH M.
Principal Investigator: WILSON, KEITH T MD
Title: REGULATION OF COLITIS BY CYCLOOXYGENASE-2
Institution: UNIVERSITY OF MARYLAND BALT PROF SCHOOL BALTIMORE, MD
Project Period: 2000/04/01-2002/01/31

DESCRIPTION (adapted from the application) Our investigations to date indicate that inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2, enzymes which produce high output nitric oxide and prostanoids, respectively, are consistently upregulated in gastrointestinal mucosal inflammation. Further, expression of these genes can regulate inflammatory pathways. We have identified important protective effects of iNOS and COX-2 in specific in vivo and in vitro models. We have also found important differences in the effects of NOS and COX-2 on inflammatory events. We have used mice with targeted deletion of either iNOS or COX-2 to assess mucosal responses to a known and common pathogen, *Helicobacter pylori*. iNOS deletion had no effect on *H. pylori* infection or the severity of gastritis. In contrast, COX-2 deletion resulted in a marked exacerbation of both acute and chronic histologic gastritis, resulted in frequent duodenal ulcer formation, which was not present in controls, and increased colonization levels of *H. pylori*. In addition, the tissues from *H. pylori*-infected COX-2-deficient [COX-2(-/-)] mice exhibited an exacerbation of the Th1-predominant, IL-12-driven dysfunctional immune response which characterizes *H. pylori* gastritis. COX-2 deletion was also associated with increased epithelial injury due to apoptosis. We have also confirmed these alterations of the immune response and apoptosis in vitro. The importance of understanding the role of COX-2 in different forms of GI mucosal inflammation is highlighted by the recent FDA approval and rapid utilization of multiple COX-2 selective inhibitors for treatment of musculoskeletal diseases. Based on our preliminary data and my long-standing interest in inflammatory bowel disease, in the current proposal we will determine the role of COX-2 in several important mouse models of colitis. We will use hapten models in which the mucosal immune response has been described, as well as two pertinent colonic infections, namely *Helicobacter hepaticus* and *Citrobacter rodentia*. These infection models were selected because of our findings in *H. pylori* gastritis and the recognition that murine IBD models appear to depend on the presence of enteric bacteria. Our specific aims are to compare COX-2(-/-) vs. (+/+) mice and wild-type mice treated with COX-2 inhibitors vs. placebo and determine the regulatory role of COX-2 in: 1. models of Th1 (TNBS) and Th2 (oxazolone) mediated colitis and 2. Colonic inflammation and injury due to *H. hepaticus* and *C. rodentia*. In both aims we will assess the effect of COX-2 on A. gross and microscopic injury; B. IL-12, Th1, pro-inflammatory and Th2 cytokine levels; and C. epithelial apoptosis. These studies are designed to establish these models and the role of COX-2 in the associated diseases, and will serve as the basis for future investigations and funding applications.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1P01ES010535-01
Program Director: MAULL, ELIZABETH A
Principal Investigator: LUBAHN, DENNIS B PHD
Title: MU CENTER FOR PHYTONUTRIENT AND PHYTOCHEMICAL STUDIES
Institution: UNIVERSITY OF MISSOURI COLUMBIA COLUMBIA, MO
Project Period: 2000/03/01-2005/02/28

The MU Botanical Center is a comprehensive research and training program that investigates the molecular mechanisms of phytochemicals and phytonutrients in human disease. The Center supports five primary research projects, pilot studies, core units and career development efforts. Project 1: "Is prostate tumor progression altered by phytonutrients?" examines the effect of phytoestrogens on prostate cancer in Estrogen Receptor (ER)-alpha and/or ER-beta KO mice in a TRAMP prostate cancer mouse model. Project 2: "Phytoestrogens and innate immunity in ER-deficient mice" determines whether phytoestrogens act in the innate immune system via ER-alpha and/or ER-beta in SCID mice. Project 3: "Treatment of cystic fibrosis (CF) with soy-derived isoflavones" is a clinical study testing the activation effects of "phytoestrogens", acting through a non-ER-mediated pathway, on the mutated CFTR protein in humans. Project 4: "Identification and characterization of botanicals" is a guide to authentication of botanicals with primary emphasis on those used in food supplements. Project 5: "Neuroprotective effects of plant polyphenols against oxidative insults" examines alternative molecular mechanisms of phytoestrogens when considered as polyphenols in neurodegenerative disease. Five Cores support these Projects and coordinate research and training activities. Core A (Administrative) provides central management. Core B (Plant) grows and classifies authenticated botanicals for use by both external scientists and Center Projects. Core C (Analytical) assesses the purity of botanical ingredients from commercial and plant sources and measures the concentration of active ingredients in cell, animal and human experiments. Core D (Animal/Nutrition) breeds, treats with botanical ingredients, and supplies the necessary transgenic and non-transgenic animals. Core E (Quantitative RNA/DNA) measures low levels of specific nucleic acids from both plant and animal sources. The Center's research investigates molecular mechanisms of the phytoestrogen and polyphenol actions in four human diseases: CF, cancer, neurodegenerative disease and immune-mediated abnormalities. The Center fosters research and training of investigators to determine the safety and efficacy of botanicals in the treatment of human disease.

Grant: 2P42ES005948-09
Program Director: THOMPSON, CLAUDIA L
Principal Investigator: SWENBERG, JAMES A DVM VET
MEDICINE:VETERINARY
MEDICINE-UNSPEC
Title: ENVIRONMENTAL EXPOSURE AND EFFECT OF HAZARDOUS CHEMICALS
Institution: UNIVERSITY OF NORTH CAROLINA CHAPEL HILL, NC
HILL
Project Period: 1992/04/01-2005/03/31

The theme of this program project is to develop the scientific bases that are necessary to implement biologically-based risk assessments for several chemicals on the National Priorities List. This will be accomplished by (1) identifying critical mechanisms related to the induction of mutations and cancer by these chemicals, (2) establishing whether or not these mechanisms follow linear or non-linear dose-response relationships between the high doses employed in animal studies and actual or modeled environmental exposures, (4) determining if sensitive populations exist that are not greater or lesser risk to selected chemicals than the general population, (5) developing new methods for determining dermal exposure to hazardous chemicals, (6) investigating factors involved in bioremediation and bioavailability, (7) evaluating mass transfer phenomena in heterogeneous multiphase subsurface systems and enhance methods of remediating such systems, and (8) developing holistochastic human exposure models that integrate space/time distributions of fate and transport with toxicokinetic models linked to human health effects to improve the assessment of risk from hazardous chemicals. The theme addresses many of the stated goals of the Superfund Basic research Program. We will accomplish our task through the investigations proposed in eight research projects and six supporting cores.

Grant: 1P42ES010337-01
Program Director: THOMPSON, CLAUDIA L
Principal Investigator: TUKEY, ROBERT H
Title: MOLECULAR MECHANISMS AND MODELS FOR EXPOSURE
Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA
Project Period: 2000/07/01-2005/03/31

Understanding the cellular and molecular mechanisms of hazardous chemicals in our environment is a critical national objective. CERCLA was established to gain knowledge on the public health risks associated with exposure to Superfund site hazardous waste. Thus, a greater understanding of the exposure pathway and the health consequences resulting from human exposure to uncontrolled hazardous waste from Superfund sites are high priorities. The goals of the UC San Diego Superfund Basic Research Program are to implement modern scientific approaches to identify and characterize genomic stress responses elicited by water borne pollutants found at Superfund sites. Because such chemical exposure leads to alterations in patterns of gene expression, defining the underlying signal transduction pathways, characterization of the targeted genetic elements and established of the functional significance of these genetic changes are essential for understanding the impact of exposure to Superfund site chemicals on human health. Our experimental strategies, which are heavily upon recombinant DNA techniques and the development of new technologies, will yield new perspectives on monitoring, remediation and mechanisms of toxicity mediated through altered gene expression and aberrant cellular signaling. To meet these goals, the UC San Diego Superfund Basic Research Program will develop a multi-disciplinary effort consisting of 7 biomedical research projects and 3 research support cores. The research will be supported in part by a Ph.D. training program. The environmental problems resulting from our location in a coastal environment and our proximity to a populated border creates unique environmental US/Mexico border issues that are of special relevance to water born pollutants. These issues will be addressed through education in the community, in the industrial sector and the EPA Region 9 level by our Outreach Program and Government liaison efforts. Investigators with complementary expertise from 10 UCSD Departments, Organized Research Units and Centers are participating in this project. Our combined efforts are anticipated to provide new insights into the molecular mechanisms that lead to illnesses arising from environmental sources and to improve our understanding of the consequences of exposure to Superfund site contaminants.

Grant: 1R01ES010182-01
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: 2000/03/01-2003/02/28

DESCRIPTION: (Adapted from the Investigator's Abstract) Botulinum toxin is the microbial agent responsible for the disease botulism. This toxin produces paralysis by inhibiting the release of acetylcholine from nerve endings. Botulinum toxin is also used clinically to treat neuromuscular diseases characterized by hyperactivity. The long-range goal of the proposed research is to define the molecular targets that mediate the mechanism of action of botulinum toxin at the clinically important toxin target tissue, the neuromuscular junction. To produce its toxic effect, botulinum toxin must first bind to specific receptors on the plasma membrane of cholinergic nerve terminals to gain entry to the intracellular space. Once inside, the toxin must find its intracellular target and inactivate it proteolytically. Although several membrane and intracellular synaptic proteins have been implicated as putative toxin receptors and intracellular targets in studies of non-target tissues, their identities at the neuromuscular junction are unknown. To begin to address these unknowns definitively, functional studies of toxin action must be combined with biochemical and molecular studies in the clinically relevant target tissue, the neuromuscular junction. To begin to resolve more fully the cellular and biochemical interactions of botulinum toxin with its target tissue, the proposed research will consist of two major elements. First, immunological detection techniques will be combined with electrophysiological recordings of endplate activity in mouse neuromuscular tissue to determine biochemically and functionally the interactions are botulinum toxin with putative intracellular target proteins. Second, the technique of molecular biology will be combined with electrophysiology to define at the molecular and functional level the specific target receptor responsible for the selective interaction of botulinum toxin with nerve terminals of the mouse neuromuscular junction. The results of the proposed studies will hopefully impact on clinical medicine by defining specific cellular targets of the neuromuscular junction that may serve as molecular templates for the development of more effective toxin antagonists and safer toxin-like therapeutic agents.

Grant: 1R21ES010776-01
Program Director: LAWLER, CINDY P
Principal Investigator: LING, ZAODUNG MD
Title: IN UTERO BACTERIOTOXINS AS A POTENTIAL ETIOLOGY FOR PD
Institution: RUSH UNIVERSITY MEDICAL CENTER CHICAGO, IL
Project Period: 2000/09/01-2002/06/30

DESCRIPTION (Taken from the Investigator's Abstract) The symptoms of Parkinson's Disease (PD) result from reduced striatal dopamine (DA) secondary to degeneration of the DA - producing cells of the substantia nigra pars compacta (SNpc). Although genetic and environmental factors are thought to be involved, the etiology of PD remains elusive. Recent preliminary findings in the investigators' laboratory show that maternal administration of lipopolysaccharide (LPS), derived from gram(-) bacteria, at embryonic (E) day 10.5 induces DA neuron loss in the fetal brain by E15. This loss is still present in the pups at postnatal (P) day 10. This suggests that prenatal gram (-) infection attenuates fetal DA neuron development. In addition, in vitro culture data as well as in vivo results demonstrate that the pro-inflammatory cytokines tumor necrosis factor (TNF-alpha) and interleukin 1B IL-1beta, which are normally induced by LPS, mediate this cytotoxic effect. This is particularly relevant since postmortem studies on PD brains reveal increased pro-inflammatory cytokines. The investigators hypothesize that gram (-) infection during pregnancy attenuates DA neuron development leaving the offspring with fewer DA neurons at birth and therefore at increased risk for PD in later life. This heretofore unexamined risk factor for PD will be explored first in a rat model (specific aim 1) and the mechanistic issues associated with LPS-induced cell death will be studied in primary mesencephalic cultures (PMCs; specific aim 2). LPS will be injected into the gravid female at E10.5 and the embryos harvested at various times thereafter through P40. TNF-alpha and IL-1Beta, IFN-gamma will be measured (ELISA) in fetal brain, and maternal blood to establish the relationship between systemic infection and fetal brain cytokine elevation. DA function (DA biochemistry by HPLC) and DA neuron counts (quantitative stereology) will be the primary outcome variables for correlational studies with the presumed cytokine alterations. Striatal glial cell line-derived neurotrophic factor (GDNF) will also be measured (ELISA) in striatal tissue. The role of TNF- alpha and IL-1Beta, and IFN-gamma in the presumed apoptotic death of DA neurons will be explored in PMCs using confocal microscopy (TH+ cells and apoptotic nuclei) as the primary outcome variable and the measurement of the pro-apoptotic Bax and the anti-apoptotic Bcl-2/Bcl-xL proteins as well as the production of nitric oxide as secondary outcome variables. The investigators anticipate that maternal LPS injection will produce a profound elevation of fetal brain cytokines that outlasts the maternal elevation and this elevation will be associated with diminution of fetal brain GDNF, DA function, and cell number. Moreover, the pro-inflammatory cytokines will kill DA neurons through apoptosis in tissue culture by decreasing Bcl-2 related proteins and by increasing nitric oxide production and that the toxic effects of LPS in culture will be attenuated by TNF-alpha and IL-1Beta antagonists. The successful implementation of these two specific aims will provide strong support for a larger proposal designed to fully explore the possible impact of gram (-) infections on DA neuron development.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2R01EY011249-05A1
Program Director: HUNTER, CHYREN
Principal Investigator: GOLDBERG, STEPHEN J
Title: EYE MOVEMENTS: FORCE, MOTION, AND ANATOMY RELATIONSHIPS
Institution: VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA
Project Period: 1995/12/06-2003/07/31

The exquisite precision with which the eyes acquire, pursue and fixate visual targets appears to stand in contrast to the more gross methods used to correct abnormalities in the system. Extraocular muscles (EOMs) may be surgically shortened or repositioned to compensate for inappropriate motor activity. Botulinum toxin type A can be injected in order to weaken a particular muscle so that it may perform better in relation to other muscles, although force changes after such injections have not been systematically studied. The predicted outcome of these measures can be unreliable and the interventions may need to be repeated in the same patient because it's often difficult to obtain the proper alignment of the eyes with a single procedure. While the clinical effectiveness of these strategies is unquestioned, there is obvious need to improve their precision and predictability. This proposal, using cats and monkeys, will primarily focus on two related aspects of eye movement control exposed by perturbing the normal system. 1) How does EOM contractile force change from 0-2 months post botulinum toxin injection and do those changes directly relate to eye displacement changes? 2) How precise are motoneuron MN firing patterns during repeated identical movements and how might that precision be altered after botulinum toxin injection? Additionally, is there a relationship between Vth nerve branching and orbital and global layer of the lateral rectus muscle? Studies of EOM electromyography (EMG), muscle immuno-histochemistry and myosin expression will be carried out concurrently with the examination of these questions. The correlative evaluation of MN firing, whole muscle plus motor unit force, muscle cytology and EMG measures with eye displacement is unique and unavailable in either normal or botulinum toxin treated motor systems. It is hoped that these studies will provide information critical to clinicians seeking to improve patient outcomes as well as basic researchers who want to understand the complex dynamics of eye movements.

Grant: 1R01EY012219-01A2
Program Director: FISHER, RICHARD S
Principal Investigator: DEAN, DEBORAH A MPH
Title: PATHOGENESIS OF CHLAMYDIAL OCULAR INFECTIONS
Institution: CHILDREN'S HOSPITAL & RES CTR AT OAKLAND, CA
OAKLAND
Project Period: 2000/05/01-2005/04/30

DESCRIPTION (Adapted from the Applicant's Abstract): Trachoma is a chronic ocular infection caused by *Chlamydia trachomatis* and is the leading cause of preventable blindness in the world. Over 500 million people are infected of whom 100 million have serious visual impairment, 9 million are blind. Blinding trachoma is predicted to increase to 12 million cases by the year 2020. Appropriate public health interventions and an effective vaccine rely on an understanding of behavioral risk factors, the organism's antigenic potential and capacity for change, its mode of transmission, and the pathogenesis of disease progression. The investigators will address the latter in this research proposal. Disease progression results in trichiasis and entropion which occur concomitantly from conjunctival scarring and are the sequelae of trachoma that lead to blindness. There are few studies that address pathogenesis. Some have evaluated local and systemic immune responses or peripheral blood mononuclear cells in proliferation assays among individuals with conjunctival scarring. Others have conducted short term studies in animal models. Still others have looked at in vitro assays of chlamydial antigens and their pathogenic effect in various cell lines and tissues. However, there are no studies examining conjunctival tissue from patients with trichiasis/entropion for latent chlamydial organisms, chlamydial hsp60, and MOMP antigens, cell types, and cytokine profiles that may contribute to the immunopathogenesis of disease. Further, no studies have prospectively looked at conjunctival mucosal reinfection rates, cytokine profiles, and chlamydial hsp60 responses at multiple time points, and correlated these with progression of clinical disease. The investigators propose to study trachoma trichiasis/entropion patients from two villages compared with age, sex, and ethnically matched, non-trachomatis scarred controls who require surgery for other reasons, and study the remaining individuals in both villages. Specific Aims: 1) Determine whether the chronic sequelae of trachoma result from latent infection and how infection correlates with cell mediated immune (CMI) responses; 2) Identify the immune mediators of trichiasis/entropion by determining cell types, hsp60 and MOMP antigens, inflammation, and cytokines present in conjunctival biopsy material; and 3) Evaluate the role of chlamydial hsp60 and CMI responses in the pathogenesis of scarring disease among trachoma patients. A greater understanding of the pathogenesis of trachoma will aid in developing public health interventions and a vaccine that can be implemented in trachoma endemic areas throughout the world.

Grant: 1R01EY012527-01
Program Director: FISHER, RICHARD S
Principal Investigator: ANSEL, JOHN C MD
Title: ROLE OF THE CD14 LPS RECEPTOR IN CORNEAL INFLAMMATION
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 2000/05/01-2004/04/30

Gram negative infections of the cornea with organisms such as *Pseudomonas* can have profound consequences for patients including bacterial keratitis which can lead to visual loss. A significant portion of the inflammatory response to *Pseudomonas* is mediated by LPS. We have recently reported that the cornea expresses one of the principal LPS receptors, CD14. The long-term goal of this project is to determine the role of the cornea itself in mediating host innate immune responses to bacterial infections. This application will test the hypothesis that corneal cells express functional CD14 receptors that, when activated by LPS, are capable of triggering the expression of proinflammatory peptides which facilitate the host resolution of gram negative corneal infections. To experimentally test this hypothesis, we will undertake the following Specific Aims: SPECIFIC AIM #1: To examine the expression and regulation of the CD14 receptor in the cornea; SPECIFIC AIM #2: To assess the functional competence of corneal CD14 receptors; SPECIFIC AIM #3: To assess the ability of CD14 activation to induce innate inflammatory responses in the cornea; and SPECIFIC AIM #4: To determine the in vivo role of the corneal CD14 receptor in mediating corneal inflammatory responses in a murine experimental model of *Pseudomonas* bacterial keratitis. To carry out these studies, human and murine corneal cells and corneal tissue will be used as well as genetically altered strains of mice in which CD14 expression is absent, diminished, or overexpressed. Cornea CD14 expression will be measured constitutively and after exposure to bacterial reagents (*Pseudomonas*, LPS, LPS/LBP) and cytokines (IL-1 and TNF α). CD14 functional activity to bacterial reagents will be measured by intracellular calcium responses, tyrosine kinase activity, and NF-kappaB activity. Corneal CD14 induced innate inflammatory responses to bacterial reagents will be determined by measuring the expression of corneal cytokines (IL-1, IL-6, and TNF α), chemokines (IL-8), and cell adhesion molecules (ICAM-1). A murine model using CD14 genetically altered animals will be utilized to assess the in vivo role of CD14 in experimental *Pseudomonas* bacterial keratitis. The activation of corneal CD14 may have both beneficial and detrimental inflammatory responses. Understanding the role of CD14 in mediating corneal innate immunity may result in novel approaches to the management of corneal infectious diseases.

Grant: 1R01EY012961-01
Program Director: FISHER, RICHARD S
Principal Investigator: O'CALLAGHAN, RICHARD J PHD
Title: MECHANISMS AND THERAPY OF BACTERIAL KERATITIS
Institution: LOUISIANA STATE UNIV HSC NEW ORLEANS, LA
ORLEANS
Project Period: 2000/05/01-2004/04/30

DESCRIPTION: (from abstract). Bacterial keratitis can result in blindness or a loss of visual acuity through a pathologic process that involves the interaction of bacteria, their products, and the host reaction to bacterial proteins. The infections are caused with almost equal frequency by Gram-positive (e.g., Staphylococcus or pneumococcus) or Gram-negative (e.g., Pseudomonas or Serratia) bacteria. While the outcomes of these bacterial infections are similar, research conducted in this laboratory has shown that the fundamental mechanisms differ dramatically. For Gram-positive bacteria, a hemolytic exotoxin is the prime mediator of tissue damage. In Pseudomonas or Serratia keratitis, bacterial proteases are essential for the intra-corneal stage of infection. The long-term goal of this research is to determine the molecular events mediating keratitis and to devise means to interrupt those reactions that result in irreversible tissue damage. The objectives of the present proposal are to determine the molecular properties of Pseudomonas protease IV, an extracellular enzyme associated with corneal virulence. This protease is produced by nearly all Pseudomonas ocular isolates and a mutant deficient in this enzyme is attenuated in animal models of keratitis. The PI's laboratory has very recently determined the DNA sequence of the gene for protease IV making possible for the first time detailed molecular biology studies. The specific aims are: 1) construct a plasmid that codes for functional protease IV and determine if the plasmid can augment the virulence of its host bacterium 2) identify the amino acids that comprise the active site of this enzyme providing the data will guide the development of a protease inhibitor, 3) prepare allele replacement mutants deficient in the protease IV gene and perform genetic rescue experiments to prove the relationship between protease IV and corneal damage, and 4) test synthetic peptides for use as immunogens in developing an immune state protective against the tissue damage associated with Pseudomonas keratitis. The results of these studies are anticipated to provide new knowledge upon which can be based chemotherapeutic or immunologic means to limit the severity of Pseudomonas corneal infections.

Grant: 1R01EY012985-01
Program Director: DUDLEY, PETER A
Principal Investigator: CALLEGAN, MICHELLE C PHD
Title: PATHOGENIC MECHANISMS OF BACILLUS CEREUS ENDOPHTHALMITIS
Institution: UNIVERSITY OF OKLAHOMA HLTH OKLAHOMA CITY, OK
SCIENCES CTR
Project Period: 2000/06/01-2004/05/31

Bacillus cereus causes the most explosive and devastating form of post-traumatic or endogenous endophthalmitis that, despite aggressive antibiotic and surgical intervention, almost always results in blindness. The regularity of treatment failures despite aggressive treatment necessitates the identification of the specific virulence factors associated with disease and characterization of the underlying pathogenic mechanisms involved. Preliminary studies employed a highly reproducible and sensitive rabbit model of experimental B. cereus endophthalmitis to analyze pathological events occurring during infection. The model was used in a comparative study of gram-positive endophthalmitis to identify the basis for strain-specific differences in virulence. Retinal damage and inflammation occurred in a pathogen-specific manner, with B. cereus endophthalmitis resulting in migration of organisms throughout the eye, significant retinal destruction, and explosive intraocular inflammation within 18 hours. Intravitreal injection of B. cereus cell walls did not affect retinal responsiveness, but induced significant intraocular inflammation. One cytolytic toxin, hemolysin BL, was found not to contribute to endophthalmitis pathogenesis. However, other as yet unidentified proteins secreted by B. cereus caused significant retinal toxicity and intraocular inflammation, paralleling that observed during a natural infection. Among bacterial causes of ocular infectious disease, B. cereus ranks at the top as one of the most virulent ocular pathogens, but ranks near the bottom in terms of understanding the host/pathogen relationship during infection. To fill this existing information gap, we propose to identify the principle factors responsible for B. cereus intraocular virulence by 1) assessing the retinal toxicity and intraocular inflammogenicity of individual B. cereus secreted products and cell wall constituents, 2) analyzing the intraocular virulence of isogenic mutants deficient in specific B. cereus toxins, and 3) examining the contribution of bacterial intraocular migration to virulence. The experiments outlined in this proposal are designed to identify primary B. cereus virulence determinants and characterize their contribution to the course and severity of disease. Identification and characterization of important virulence factors will provide the basis on which information-based therapeutic agents are developed in order to prevent vision loss during B. cereus endophthalmitis.

Grant: 2R01GM013626-32A1
Program Director: RHOADES, MARCUS M.
Principal Investigator: GALLANT, JONATHAN A PHD
Title: CONTROL OF CELL GROWTH
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 1979/05/01-2004/01/31

In translating the genetic code, ribosomes normally move along the messenger RNA reading one codon at a time. Ribosomes maintain a reading frame by moving in three-base steps. However, alternative forms of movement, can produce single-base changes in reading frame either backward (to the left, toward the 5' end of the message) or forward (to the right, toward the 3' end of the message). Such programmed changes producing two different essential translation products, have been found particularly in the translation of viral RNAs, but also occasionally in chromosomal genes. This proposal focuses on three categories of unconventional ribosome movement. First is the stimulation of both leftward and rightward frameshifting at so-called "hungry" codons where the ribosome stalls due to a limiting supply of cognate aminoacyl-tRNA. Second is the stimulation of frameshifting in stationary phase or during carbon/energy source downshift which is independent of frameshifting at "hungry" codons. Third, is the recently discovered phenomenon of ribosome leaping at hungry codons. In this case, the ribosome-peptidyl-tRNA complex skips a long stretch of mRNA and then resumes translation elongation at a downstream site. This is especially interesting because of the possibility that pt-tRNA can remain stably bound in the ribosomal P site without directly engaging mRNA during the process of ribosome movement (by whatever mechanism) to the so-called landing site at which elongation resumes. Because starvation conditions and the occurrence of a hungry codon may be frequent in nature, these phenomena are important to an understanding of translation and its control.

Grant: 2R01GM018568-28
Program Director: GREENBERG, JUDITH H.
Principal Investigator: LOSICK, RICHARD M PHD
GENETICS:BIOCHEMICAL/M
LECULAR
Title: RNA POLYMERASE AND BACTERIAL DIFFERENTIATION
Institution: HARVARD UNIVERSITY CAMBRIDGE, MA
Project Period: 1976/02/01-2004/01/31

The objectives of this project are to understand the control of gene transcription during sporulation in *Bacillus subtilis* and how the expression of sporulation genes both directs, and is kept in register with, the course of morphogenesis. Sporulation involves the formation of a polar septum, which divides the developing cell into a forespore and a mother cell. Initially, the two cells lie side-by-side but later the forespore is engulfed by the mother cell. Evidence indicates that the polar septum is an organelle that is intimately involved in the establishment of cell-specific gene transcription. Differential transcription is governed initially by the action of transcription factor sigmaF in the forespore and sigmaE in the mother cell. After engulfment, sigmaF and sigmaE are replaced by sigmaG and sigmaK, respectively. Experiments will be carried out to understand how septum formation switches from a medial to a polar position. A pathway is known that links the activation of sigmaF to the polar septum. Experiments will be carried out to understand the roles of a septum-associated phosphatase and the proteolysis of an antisigma factor in the activation of sigmaF. The sigmaE factor is derived from pro-sigmaE, which associates with the septum prior to undergoing activation by proteolytic processing. Experiments will be carried out to understand the role of a septum-associated protein in restricting sigmaE to the mother cell. The sigmaK factor is derived from pro-sigmaK, whose activation is governed by an intercellular pathway that is coupled to the action of sigmaG. Experiments will be carried out to determine how the components of the pathway mediate pro-sigmaK processing in response to a signal from the forespore. Spore formation involves the assembly around the forespore of a morphogenetic protein that is produced under the control of sigmaE. Experiments will be carried out to understand the localization of this protein and its role in recruiting coat proteins and triggering cortex synthesis. These objectives address basic questions of differentiation and morphogenesis that are common to developing systems of many kinds, including complex systems of normal and abnormal development in higher organisms.

Grant: 2R01GM019043-29
Program Director: SHAPIRO, BERT I.
Principal Investigator: INOUYE, MASAYORI PHD
GENETICS:BIOCHEMICAL/M
LECULAR
Title: STRESS RESPONSE AND ADAPTATION IN ESCHERICHIA COLI
Institution: UNIV OF MED/DENT NJ-R W JOHNSON MED PISCATAWAY, NJ
SCH
Project Period: 1987/01/01-2003/12/31

Stress response and adaptation is the basic principle for microorganisms to survive in their natural habitats. Whether they are living in host organisms or directly exposed to nature, they are all equipped with a large number of defense systems against stresses. This proposal is focused on two major stress- response systems in Escherichia coli. First, the molecular mechanisms of stress response through transmembrane histidine kinases, and secondly, the molecular mechanisms of cold-shock response and adaptation. The understanding of these two major stress response systems in E. coli, one through membrane receptors and the other by direct biophysical effects of temperature changes on cellular components will have important implications in studying stress responses in wide varieties of living organisms. We have recently determined the first ever three-dimensional solution structure of the histidine kinase domain of EnvZ, an osmosensor that regulates porin gene expression (ompF and ompC). We also demonstrated that dimerization of histidine kinases is obligatory for its biological function. In this proposal, we attempt to fully decipher the molecular mechanism of (a) the kinase (autophosphorylation and OmpR kinase) and phosphatase (phospho- OmpR dephosphorylation) activities; (b) the transduction of signal across the membrane of ligand binding that regulates histidine kinase function; (c) transcriptional activation and repression of the two porin genes through phospho-OmpR. In the cold-shock project, we have identified CspA, a major cold-shock protein functioning as an RNA chaperone, and 8 other CspA homologues, CspB to CspI. We will continue to elucidate the roles of individual Csp proteins in this nine-membered family. In particular, we will take advantage of a quadruple deletion mutant (DcspA DcspB DcspG DcspE) of E. coli, which is cold- sensitive. This quadruple mutant will allow us to characterize the roles of individual Csp proteins and other functionally related proteins by complementation experiments. We will also continue to investigate the regulatory mechanisms of the cspA expression at low temperature at the level of transcription, mRNA stability and translation initiation. The proposed research will provide important insight into the fundamental principles of stress response and adaptation in prokaryotes.

Grant: 2R01GM020011-29
Program Director: JONES, WARREN
Principal Investigator: WALSH, CHRISTOPHER T PHD
Title: ENZYMATIC REACTION MECHANISMS
Institution: HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA
Project Period: 1987/09/30-2003/12/31

The goals of this proposal are the study of enzymatic reaction mechanisms. In this project period the focus is on the reaction mechanisms of two kinds of enzyme systems involved in the biosynthesis of peptide antibiotics. The first is the enzymatic heterocyclization machinery involved in the conversion of the 69aa Microcin A protein, an antibiotic precursor, to Microcin B17, an antibiotic targeted against E.coli DNA gyrase, in which 14 residues (six gly, four ser, four cys) have been posttranslationally modified to four thiazole and four oxazole rings, essential for antibiotic activity. The second goal is analysis of the enzymatic strategies used by multimodular enzymes that make peptide bonds nonribosomally, e.g. in the biosynthesis of peptide antibiotics and iron-chelating siderophores. The example to be studied is the four enzyme system, Ent B,D,E,F, responsible for formation of the E. coli iron chelator enterobactin. In particular the EntF enzyme has four domains (condensation, adenylation, peptidyl carrier protein and thioesterase) whose functions in assembly of the (dihydroxybenzoyl)-serine trilactone, enterobactin, will be analyzed in terms of covalent priming, initiation, elongation, and termination strategies.

Grant: 2R01GM023719-25
Program Director: WOLFE, PAUL B.
Principal Investigator: MODRICH, PAUL L PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: MOLECULAR MECHANISM OF DNA-PROTEIN INTERACTION
Institution: DUKE UNIVERSITY DURHAM, NC
Project Period: 1977/08/01-2004/07/31

DESCRIPTION: (adapted from the application abstract) Mismatch repair plays a major role in genome stabilization, and available information indicates that the substrate specificity and mechanism of the reaction have been highly conserved during evolution. The *Escherichia coli* methyl-directed pathway, which is the best understood of the known mismatch repair systems, provides a paradigm for study of the mechanism of this complex reaction. Dr. Paul Modrich and his colleagues have identified eleven activities that are involved in mismatch repair, including MutS, MutL, MutH, DNA helicase II, and DNA polymerase III holoenzyme. This application addresses several features of the mechanism of this interesting reaction: (i) MutS and its eukaryotic homologs are responsible for mismatch recognition, but also possess a slow ATPase that is required for MutS function in mismatch repair. It is now clear that these proteins leave the mismatch in an ATP-dependent reaction to move along the helix contour, an effect that is believed to play an important role in the coupling of mismatch recognition to the recognition of a strand signal elsewhere on the helix that confers strand specificity on the reaction. However, the mechanism of this movement is controversial. They plan experiments that they hope will resolve this question with respect to the bacterial protein. (ii) In collaboration with the laboratory of Lorena Beese, they are pursuing structural analysis of bacterial MutS in order to clarify the basis of its ability to recognize mismatched base pairs. (iii) They showed that MutS and MutL load DNA helicase II at the strand break introduced by MutH, the key step in initiation of mismatch-provoked excision. They have also shown that MutL functions as an activator of the helicase. They hope to clarify the molecular basis of this activation. (iv) Several protein-DNA assemblies have been documented during the course of the methyl-directed reaction, although their nature has only been addressed in qualitative terms. They hope to establish the molecular composition of these complexes. This phase of the work will also address the basis of the specific requirement for DNA polymerase III holoenzyme in the repair synthesis step of the reaction.

Grant: 2R01GM023913-22
Program Director: PREUSCH, PETER C.
Principal Investigator: FULCO, ARMAND J PHD
Title: SOLUBLE CYTOCHROME P450 HYDROXYLASES FROM BACTERIA
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 1977/08/01-2002/03/31

DESCRIPTION (Adapted from the applicant's abstract): The long-term objective of the research project is the elucidation of the biochemistry, molecular biology, and functions of the cytochrome P450 enzymes induced by barbiturates in the bacterium, *Bacillus megaterium*. Goals include the determination of the mechanism for barbiturate-mediated induction and structure-function relationships of these P450s in the bacterium and application of the findings to study analogous barbiturate-inducible systems in mammalian liver. This laboratory has identified and described 3 barbiturate-inducible P450s from *B. megaterium*. One, P450BM-3, contains both P450 and a NADPH:P450 reductase in the proteolytically separable domains of a single, soluble, 119 kDa polypeptide. It functions as a fatty acid monooxygenase independently of any other proteins and resembles liver microsomal systems in organization, sequence identity and mode of induction. Its gene has been cloned and sequenced (including the regulatory region), as has the complete gene for P450BM-1. P450BM-2 has not yet been cloned. Specific aims for the next 4 years include (1) the cloning (using DNA-probe hybridization screening techniques), sequencing and expression of the P450BM-2 gene, (2) continuation of our efforts to identify and characterize all of the barbiturate-responsive transcription factors involved in the regulation of the *B. megaterium* P450s and to elucidate, at the gene level, the roles these transcription factors play in the normal and barbiturate-mediated regulation of expression of the three *B. megaterium* P450 genes, (3) comparative studies, utilizing recombinant DNA and protein-characterization techniques, of the barbiturate-mediated induction mechanisms of the bacterial and the analogous mammalian liver P450s including the cloning of the analogous (barbiturate-responsive) mammalian transcription factors, (4) functional characterization of rat regulatory proteins (currently Barbie box binding proteins) involved in transcription of barbiturate-inducible genes by co-transfection of primary rat hepatocytes with vectors expressing these proteins and reporter constructs containing the promoter sequences of the genes they regulate (currently rat CYP2B1) to drive expression of a luciferase gene, (5) the continued delineation of the structure-function relationships of the *B. megaterium* P450s including substrate-binding, specificity of oxygenation and electron transfer) utilizing enzymological techniques and site-specific mutagenesis in conjunction with X-ray crystallography. The health-related implications of the proposed research include an increased understanding of the roles that liver cytochrome P450 enzymes and their inducers (including tumor promoters such as the barbiturates) play in carcinogenesis and in the development of tolerance to therapeutic drugs.

Grant: 2R01GM027673-19
Program Director: SOMERS, SCOTT D.
Principal Investigator: COOK, JAMES A
Title: ROLE OF EICOSANOIDS IN SHOCK
Institution: MEDICAL UNIVERSITY OF SOUTH CAROLINA CHARLESTON, SC
Project Period: 1980/04/01-2004/06/30

Description: (Adapted from the applicant's abstract). Septic shock occurs in more than 500,000 patients each year in the United States, nearly half of who die. Activation of macrophages by microbial products such as Gram-negative bacterial lipopolysaccharide (LPS) leads to the release of inflammatory mediators that are a major factor in the systemic inflammatory response of sepsis. LPS binds to several macrophage membrane receptors, which activate multiple signaling cascades. Potential interactions of the signal transduction pathways activated by LPS via its receptors, and post-receptor transducers, remain to be clearly defined. Post-receptor coupling to specific guanine nucleotide regulatory (G) proteins constitutes a novel pathway of LPS activation. These signal transduction pathways may be distinct from LPS signaling events leading to nuclear translocation of the transcription factor NF κ B. LPS tolerance induced by pre-exposure to low concentrations of LPS increases resistance to LPS lethality and alters NF κ B signal transduction and mediator release. Altered macrophage signal transduction is a hallmark of tolerance. In LPS tolerant macrophage, G protein-coupled signaling pathways are altered. The specific LPS receptors or signal transduction pathways that induce tolerance are not known. However induction of LPS tolerance has been linked to changes in the NF κ B p50 subunit composition. The major hypothesis is that LPS tolerance alters G protein regulated signal transduction pathways through NF κ B initiated transcription-dependent events. Specific aims proposed to test this hypothesis will: 1) determine G α protein regulated pathways of activation by LPS and their alterations in LPS tolerance and 2) determine the role of NF κ B-initiated transcription events in LPS tolerance as a mechanism for altered G protein signal transduction. In the first Specific Aim genetic, pharmacologic, and molecular approaches will delineate potential links between specific LPS receptors and G protein regulated pathways activated by LPS. Proximal signal changes in the G protein coupled pathways induced by LPS tolerance will be characterized in cell lines, and in primary murine and rat peritoneal macrophages and human monocyte. The effect of inhibiting G protein regulated signaling in murine sepsis models will be tested. Specific Aim 2 will investigate links between NF κ B and tolerance-induced changes in the G protein coupled signaling. We will use mice genetically deficient in NF κ B (p50) and cellular over-expression of NF κ B (p50). Induction of phosphatases by NF κ B will be examined as a mechanism of altered signal transduction in LPS tolerance. Delineation of cellular mechanisms responsible for LPS tolerance may provide a rational basis for therapeutic interventions in sepsis.

Grant: 2R01GM029812-25A1
Program Director: EDMONDS, CHARLES G.
Principal Investigator: MC CLOSKEY, JAMES A PHD
CHEMISTRY:CHEMISTRY-
UNSPEC
Title: STRUCTURAL STUDIES OF NUCLEIC ACID CONSTITUENTS
Institution: UNIVERSITY OF UTAH SALT LAKE CITY, UT
Project Period: 1978/05/01-2003/12/31

Post transcriptionally modified nucleotides in RNA are known to play a variety of functional roles, including stabilization of secondary and tertiary structure and enhancement of fidelity of intermolecular interactions in protein synthesis. By far, the greatest amount of information has been derived from studies of tRNA, although the details of these roles are in general not well understood. By contrast much less is known regarding structure-function relationships for post-transcriptional modifications in ribosomal RNAs even though both large and small subunit rRNAs are now known to play direct roles in protein synthesis. It is proposed to apply new electrospray ionization LC/MS and LC/MS/MS based protocols to identify modified nucleotides and place them at precise sites in sequences of RNA from small and large ribosomal subunits, and of tRNAs. The proposal is focused on ribosomal RNAs and transfer RNAs of thermophilic bacteria and archaea, as models of understanding natural structural motifs for RNA stabilization. Modified oligonucleotides selected from the RNA modification maps determined will be synthesized for study of their biophysical properties compared with those of the corresponding unmodified sequences. These studies contribute to a clearer understanding of ribosome structure and function during protein synthesis, and of structural motifs that influence RNA stability. These issues are of potential importance in the design of antisense- and ribozyme-based therapeutic agents, in understanding the basis of resistance to ribosome-targeted antibiotics, and in expanding the limited knowledge based of natural modifications in RNAs involved in translation.

Grant: 2R01GM030717-17
Program Director: TOMPKINS, LAURIE
Principal Investigator: GOLDFARB, ALEXANDER PHD
Title: STRUCTURE AND FUNCTION OF RNA POLYMERASE IN E COLI
Institution: PUBLIC HEALTH RESEARCH INSTITUTE NEWARK, NJ
Project Period: 1983/03/01-2004/02/29

RNA polymerase (RNAP) is the enzyme that catalyzes the first step in gene expression--the transcription of genetic information into messenger RNA. As such it is the principal target for factors regulating gene expression and its basic functions and structural features are highly conserved evolutionarily. The broad goal of this project is the understanding of the molecular mechanism of RNAP function and regulation. In collaboration with X-ray crystallographers, Dr. Goldfarb would like to understand RNAP structure in relation to its interactions with DNA, with nucleotide substrates, and with its RNA product. Single amino acid substitutions will be generated in the cloned genes, rpoB and rpoC, which specify the two largest subunits of RNAP holoenzyme. The mutations will be characterized using a variety of in vitro assays to determine which discrete activities of the enzyme are affected. In addition, the PI proposes to develop a series of engineered nucleic acid model ligands that bind stably to RNAP to form complexes analogous to those formed during transcription elongation; these ligands will be used for co-crystallization with the enzyme to refine the structural models and ascertain differences in conformations assumed by the enzyme at discrete steps in the transcription elongation reaction.

Grant: 2R01GM031685-18A1
Program Director: WOLFE, PAUL B.
Principal Investigator: KHAN, SALEEM A PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: PLASMID PT181 DNA REPLICATION IN STAPHYLOCOCCUS AUREUS
Institution: UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PA
PITTSBURGH
Project Period: 1982/07/01-2004/01/31

DESCRIPTION (adapted from the investigator's abstract): Staphylococcus aureus is an important human pathogen and a reservoir for a large number of plasmids that encode resistance to many commonly used antibiotics. A large group of multicopy plasmids in S. aureus encode resistance to a single antibiotic and replicate by a rolling-circle (RC) mechanism. While several RC plasmids found in S. aureus have a narrow host range, many are able to be stably maintained in a wide range of Gram-positive bacteria. The long term goals of this proposal are to understand the molecular basis for stable replication of RC plasmids in S. aureus and other Gram-positive bacteria, using pT181 and related plasmids as model systems. We will carry out site-directed mutagenesis to understand the role of ssoA-type origins in lagging strand synthesis of RC plasmids, and the basis of their host-specific function. The molecular basis for broad host range function of the ssoU-type origins will also be investigated. The effect of RepC and the pT181 origin on the helicase activity of PcrA will be determined. These studies are expected to provide information on the role of this interaction in the initiation and termination of plasmid pT181 RC replication. The RepC protein acts as a dimer and has DNA binding and nicking-closing domains. Purified heterodimers of wild-type and mutant RepC proteins will be used to identify the role of each monomer during the initiation and termination of pT181 replication. A new series of experiments utilizing fluorescence microscopy will be initiated to determine whether the pT181 plasmid replicates at a discrete site in the cell termed the "replication factory." We have obtained co-crystals of RepC bound to its specific binding site and will continue efforts to obtain larger crystals of RepC and RepC-DNA complex and determine their structure by X-ray crystallography. These studies are expected to provide information on the replication and maintenance of drug resistance plasmids in S. aureus and other Gram-positive bacteria that replicate by an RC mechanism.

Grant: 2R01GM033471-16
Program Director: ANDERSON, JAMES J.
Principal Investigator: HULETT, MARION F PHD
MICROBIOLOGY:MICROBL
BIOCHEMISTRY
Title: B.SUBTILIS PHO REGULON SIGNAL TRANSDUCTION NETWORK
Institution: UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL
Project Period: 1985/01/01-2004/06/30

Two-component signaling is the principal form of signal transduction in prokaryotes with distinctive examples in eukaryotes. At the end of logarithmic growth, the "transition state" Bacillus cell receives multiple signals from the environment that are simultaneously reporting conditions such as temperature, cell density, nutrition availability and oxygen tension. These signals are processed to determine the most appropriate gene expression and metabolic response for survival. The characterization of the B. subtilis Pho signal transduction network has led to the hypothesis that "The processing of the multiple signals is accomplished by regulatory networks involving multiple two-component systems that function to establish dependencies or hierarchies between systems. This overlap between regulons provides a mechanism for signal integration and signal input experienced by the organism at any one time." The proposal focuses on the B. subtilis Pho regulon signal transduction network to understand how multiple two-component systems interact to participate in a signal transduction network. The PI is planning to: 1) determine if overlap of regulons occurs at the level of transcription of the phoPR operon encoding the primary Pho two-component regulators by asking what proteins control the expression of each of the four phoPR promoters; 2) determine how two two-component regulators, ResD and PhoP, interact, such that each is essential but not sufficient for transcription of an operon encoding one of the regulatory pairs, ResD and ResE; 3) identify additional regulators proposed to assist the catalytic domain of PhoR, making that domain sufficient to control the phosphate deficiency induction of the Pho regulon; 4) determine if differences in domain interaction among response regulators explains differences in target DNA-response regulator interactions or protein oligomeric state with respect to phosphorylation.

Grant: 2R01GM033992-16A1
Program Director: WOLFE, PAUL B.
Principal Investigator: KAGUNI, JON M PHD
BIOCHEMISTRY:NUCLEIC
ACID
Title: MECHANISMS OF ESCHERICHIA COLI CHROMOSOMAL REPLICATION
Institution: MICHIGAN STATE UNIVERSITY EAST LANSING, MI
Project Period: 1984/07/01-2004/03/31

The E. coli genome is a circular duplex DNA molecule containing a single replication origin, oriC. DNA replication that starts at this site is initiated by the binding of DnaA protein to specific sequences termed DnaA boxes. A series of discrete events follow to establish the protein machinery at each replication fork for bidirectional replication fork movement. This process to produce two progeny DNA molecules is coordinated to cell growth and is regulated at the step of initiation of chromosomal DNA replication. The long term objectives of this research are to understand the process of initiation of E. coli chromosomal DNA replication at the biochemical level, and to determine how this process is regulated. Our recent results indicate that DnaA protein interacts directly with DnaB in its entry at oriC. Furthermore, DnaA protein plays an active role in the loading of DnaB onto the DNA. In the next funding period, we will pursue several aims to establish how an intermediate of the initiation process termed the prepriming complex is assembled and how these proteins interact. Specific aims are: i) to determine the stoichiometry of DnaA, DnaB and DnaC in the prepriming complex formed at oriC and to investigate possible mechanisms that control the entry of DnaB, ii) to identify where DnaB enters at oriC, iii) to identify specific amino acids of DnaA that are important for binding to DnaB, iv) to determine of the physical form of DnaB protein that interacts with DnaA protein, v) to identify the regions of DnaA protein involved in self-aggregation, vi) to identify and characterize other proteins that bind to DnaA protein as an affinity ligand, and vii) to identify amino acids of DnaC that are important for interaction with DnaB. These studies will provide further insight on the role of DnaA protein in the initiation process. Furthermore, the biochemical events in the initiation process in this model system may have similarities to the mechanism of initiation of chromosomal replication in other organisms.

Grant: 2R01GM035215-13A1
Program Director: SHAPIRO, BERT I.
Principal Investigator: CAFISO, DAVID S
Title: MOLECULAR MECHANISMS OF MEMBRANE TRANSPORT
Institution: UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA
CHARLOTTESVILLE
Project Period: 1985/09/06-2004/07/31

Active and passive transport are critical process for normal cell metabolism, including the maintenance of ion-gradients, osmotic balance, membrane potentials, and apoptosis. In spite of their widespread importance, the molecular mechanisms that lead to transport have not been characterized, which is in part due to the difficulty associated with obtaining structural information on membrane proteins. The work that is described in this proposal is directed at investigating the mechanisms of action of two transport systems. BtuB is an outer membrane transport protein for vitamin B12 found in gram negative bacteria, and it obtains its energy for transport by coupling to the inner membrane protein TonB. Using site-directed spin-labeling and EPR spectroscopy, we will test proposals for the molecular mechanisms of transport in BtuB. This class of membrane proteins is of fundamental interest because it is the only class of membrane active transport proteins for which high-resolution structural models have been obtained; as a result, they are likely to be the first active transport systems for which detailed molecular mechanisms will be obtained. In addition to the accumulation of nutrients, this class of proteins functions in the uptake of bactericidal agents, such as colicins, phages and small molecule antibiotics. The widespread importance of these TonB dependent systems for bacterial function makes them probable targets for the development of new classes of antibiotics. A second system that will be studied is alamethicin, a peptide antibiotic that forms voltage-dependent ion channels in bilayers. Voltage- dependent events in membrane proteins are also of widespread importance, but have not been characterized at a molecular level. Alamethicin belongs to a larger class of membrane active peptides having important antibiotic, fungicidal, hemolytic and tumoricidal activities. In addition to its voltage-dependence, alamethicin has received attention because it appears to be selective towards certain organisms. In the work proposed here we will develop a method to apply electric fields across vesicle and supported bilayers and will use NMR, EPR and FTIR to test mechanisms for the voltage-dependence in this peptide. We will also test mechanisms that could account for the selectivity of this antibiotic against certain membranes.

Grant: 2R01GM035682-15A1
Program Director: ANDERSON, JAMES J.
Principal Investigator: BLATTNER, FREDERICK R PHD
Title: COMPLETE DISSECTION OF ESCHERICHIA COLI GENOME
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 1985/04/01-2003/02/28

Abstract Text Not Available

Grant: 2R01GM035695-14A1
Program Director: WOLFE, PAUL B.
Principal Investigator: MCHENRY, CHARLES S
Title: ASSEMBLY OF REPLICATIVE COMPLEXES
Institution: UNIVERSITY OF COLORADO DENVER/HSC AURORA, CO
AURORA
Project Period: 1985/12/01-2004/08/31

The DNA polymerase III holoenzyme of *E. coli* is a prototypical replicative complex, exhibiting properties in common with other cellular replicases, including a high rate of processive elongation and the ability to interact with other proteins at the replication fork, establishing the communication channels necessary to coordinate the events required for efficient chromosomal replication. A key component of all cellular replicases is a multi-subunit assembly of homologous proteins that require ATP to assemble a 'sliding clamp processivity factor' onto primer termini. In *E. coli*, this function is served by the DnaX complex, DnaX3-delta-delta-chi-psi. The *dnaX* gene of *E. coli* encodes two distinct products: tau, the full-length translation product and gamma, a shorter protein that arises by translational frameshifting. In spite of gamma and tau being found together within holoenzyme, they do not readily form mixed complexes in vitro or when overexpressed in vivo. During the last grant period, we have determined the limits of five domains of the tau DnaX protein, the three domains of the homologous delta-prime subunit, and have defined the subunit interactions of each domain. During the next grant period, we will determine the factors required for proper DnaX complex assembly using an in vitro assay we have recently developed. We will refine our understanding of the subunit interface domains down to the amino acid level. Lastly, we have developed a system that permits individually assessing the occupancy of each polymerase site of the dimeric holoenzyme with primer-templates. This system will be used to determine whether the internal DnaX clamp loader can assemble beta2 for both strands of DNA at the replication fork, and to further investigate our asymmetric dimer hypothesis. These studies are expected to significantly improve our understanding of the structure and function of this important and highly conserved enzyme system, to provide insight into how living systems assemble their multisubunit complexes that load processivity factors onto DNA, and to help us understand the functional advantages of such complexes.

Grant: 2R01GM036526-14A1
Program Director: SHAPIRO, BERT I.
Principal Investigator: MC MACKEN, ROGER L PHD
BIOCHEMISTRY:NUCLEIC
ACID
Title: HOST-VIRUS INTERACTIONS IN INITIATION OF DNA REPLICATION
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 1986/04/01-2004/07/31

We propose to continue our studies aimed at elucidating the mechanisms by which the *E. coli* DnaK (Hsp70), DnaJ (Hsp40), and GrpE heat shock proteins cooperatively function as molecular chaperones. Homologues of these highly conserved Hsp70 and Hsp40 chaperones play vital roles in cells of all living organisms through their action in many aspects of protein metabolism, including protein folding, cellular growth control, neurotransmission, steroid receptor maturation, tumor suppression, and oncogenesis. We will continue our ongoing kinetic analysis of the intrinsic ATPase of DnaK with the goal of exploring how the coupling of the ATPase cycle to the binding and release of polypeptide substrates is affected by (i) wild type and mutant DnaJ cochaperones, (ii) the affinity of nucleotides for DnaK, or (iii) small molecule effectors such as inorganic phosphate and monovalent cations. In a parallel approach, we will construct a series of site-specific DnaK and DnaJ substitution mutants that will have single tryptophan residues inserted at various strategic locations. These locations will be chosen with the anticipation that the fluorescence properties of the substituted tryptophan will be sensitive to structural or conformational changes that occur during interaction of DnaK with DnaJ, GrpE, or nucleotides or during interaction of either DnaJ or DnaK with polypeptide substrates. Fluorescence changes occurring in these substituted proteins will be monitored by stopped-flow kinetics, which will facilitate detailed analysis of individual steps in the chaperone reaction cycle. We will also use surface plasmon resonance technologies to characterize further the interaction of DnaK with wild type and mutant forms of DnaJ and to probe the mechanisms involved in the selection of polypeptide substrates for DnaK. Finally, we will investigate the molecular mechanisms involved in a prototypical chaperone-mediated protein remodeling reaction. Specifically, we shall study the DnaJ- and DnaK-mediated disassembly of a highly stable nucleoprotein complex, formed on short single-stranded oligonucleotides, that contains the bacteriophage lambda O and P replication proteins and the *E. coli* DnaB helicase. Radiolabeled proteins and transient kinetic assays will be used to more precisely describe the nature of reactants, intermediates, and disassembly products.

Grant: 2R01GM036718-14
Program Director: ANDERSON, JAMES J.
Principal Investigator: SONENSHEIN, ABRAHAM L PHD
MICROBIOLOGY:MICROBL
PHYSIOLOGY
Title: REGULATION OF GLUTAMATE SYNTHESIS IN BACILLUS SUBTILIS
Institution: TUFTS UNIVERSITY BOSTON BOSTON, MA
Project Period: 1986/09/01-2004/07/31

The long-term goal of this project is to understand at the molecular level the mechanisms that control synthesis and activity of the enzymes that convert acetyl CoA and oxaloacetate to glutamate in *Bacillus subtilis*. This pathway, which is critical for generation of energy, reducing power, and biosynthetic building blocks, is also the junction between carbon and nitrogen metabolism and plays a key regulatory role in bacterial differentiation. Previous work on this project has led to the discovery or identification of seven proteins (CcpC, CcpA, CodY, GltC, GltR, TnrA, and RocR) that participate in regulation of the genes that encode these enzymes. The specific aims of the present proposal are to uncover the molecular mechanisms by which several of these regulatory proteins control transcription of the citrate synthase, aconitase, isocitrate dehydrogenase, glutamate synthase, and glutamate dehydrogenase genes and to discover the effector molecules that control the activities of some of the regulatory proteins. The *B. subtilis* system is the primary paradigm for studies of Gram-positive bacteria and of prokaryotic differentiation. Fundamental studies of *B. subtilis* gene expression, regulation of metabolism and response to the environment are highly informative about the biology of related pathogenic bacteria and provide a means of studying issues of universal biological importance in an organism that is easily manipulated physiologically and genetically.

Grant: 2R01GM036810-15
Program Director: CHIN, JEAN
Principal Investigator: HERZFELD, JUDITH PHD CHEMISTRY:PHYSICA
Title: NMR STUDIES OF BIOLOGICAL MEMBRANES
Institution: BRANDEIS UNIVERSITY WALTHAM, MA
Project Period: 1985/09/30-2004/03/31

Description: (Verbatim from the applicant's abstract) A distinctive feature of living cells is the facility with which they convert energy from one form to another. Central among these energy transactions is the active transport of ions across cell membranes. In recent years, significant progress has been made in elucidating the structures of various ion pumps. However, the molecular mechanisms by which vectorial ion motion is enforced remain unknown. Our long-term goal is to understand how proteins carry out this essential aspect of energy transduction. A particularly propitious system for this purpose is the light-driven proton pump, bacteriorhodopsin. Key steps in the photocycle have been identified and the structure of the resting state has been established by diffraction methods (with the exception of a few disordered, packing sensitive, or hydration sensitive regions). Our goal is to obtain a detailed picture of the active site of the molecule as it evolves through the critical steps of the photocycle. In particular, we are concerned with the structural changes that occur around the Schiff base of the retinal chromophore while it is deprotonated (i.e., during the M stage of the photocycle), because these changes prevent the proton that is released to the extracellular side from returning when the Schiff base reprotonates. Thus we are looking specifically for a switch in the connectivity of the deprotonated Schiff base between transport pathways on the two sides of the membrane and clues to the source of the irreversibility of this switch. Since our work was last funded, we have identified early and late M intermediates and learned how to trap them at levels suitable for solid state NMR studies. In addition, the last few years have seen a dramatic expansion of the range and power of solid state NMR techniques and we have developed procedures for new variations in the isotopic labeling of bacteriorhodopsin that will allow us to take advantage of the new spectroscopy. Bringing these elements together, we will characterize the spatial relationships and chemical exchange connectivities that define the active site and how they change in the critical photocycle intermediates.

Grant: 2R01GM037704-13A1

Program Director: SOMERS, SCOTT D.

Principal Investigator: TAYLOR, FLETCHER B MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC

Title: MECHANISMS OF MICROVASCULAR THROMBOSIS IN BABOONS

Institution: OKLAHOMA MEDICAL RESEARCH OKLAHOMA CITY, OK
FOUNDATION

Project Period: 1986/12/01-2003/03/31

DESCRIPTION: (Investigator's abstract) Previous studies have documented the role of inflammatory mediators (e.g., TNF, IL-6), hemostatic mediators (e.g. tissue factor/VIIa), hemostatic regulators (e.g. proteins C and S), and hemostatic modulators (e.g C4b binding protein) in driving and controlling the baboon response to *E. coli*. The anti-inflammatory effects of the hemostatic regulators particularly those of the protein C system, and the recent discovery of the involvement of the endothelial protein C receptor (EPCR) and thrombin activated fibrinolytic inhibitor (TAFI) in this system, have led us to study their contribution to its anti-inflammatory and anticoagulant properties. EPCR is the newest member of the endothelial/protein C network and it, like protein C itself, is essential in controlling the response to *E. coli*. The form of EOCR is unique in that while it inhibits protein C anticoagulant activity in vitro it also inhibits tight neutrophil/endothelial cell interactions under flow conditions. We plan to examine the pathophysiologic relevance of these in vitro observations in primate non-inflammatory (thrombin) and inflammatory (*E. Coli*) models of DIC. TAFI, the newest member of fibrinolytic inhibitors which are connected to the protein C network, also is unique in that, (1) while it is activated by the thrombin/thrombomodulin complex, its formation is down regulated by activated protein C which also is generated by this complex, (2) it is a carboxypeptidase with potential anti-inflammatory as well as antifibrinolytic activity. Again the relevance of these in vitro observations is unknown, nor is it known under what pathophysiologic conditions TAFI comes into play. We plan to examine TAFI function in the primate models noted above as well as in the C4bBP/sublethal *E. coli* model of microvascular thrombosis. Finally, we recently found that the endothelial/protein C network of diabetic baboons failed to mount an anticoagulant response to fXaPCPS. It was only the fibrinolytic "back up" response that saved these animals. We plan to determine which components of the protein C system are responsible for this failure and in addition to assess what effect this diabetic deficiency has on the endothelial susceptibility to inflammatory stress (e.g., TNF, IL-6).

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2R01GM038032-14
Program Director: SOMERS, SCOTT D.
Principal Investigator: LANG, CHARLES H
Title: CYTOKINE REGULATION OF IGF SYSTEM DURING INFECTION
Institution: PENNSYLVANIA STATE UNIV HERSHEY HERSHEY, PA
MED CTR
Project Period: 1987/04/01-2004/06/30

Muscle wasting remains a major cause of morbidity and mortality in patients after injury and infection. The working hypothesis to be tested by the proposed research is that Components of the insulin-like growth factor (IGF) system, regulated by the overproduction of endogenous cytokines, are capable of impairing muscle protein synthesis in response to a septic challenge. To address this hypothesis, the research has the following specific aims: (1) to determine the role of sepsis and TNFalpha in modulating in vivo gene expression of myostatin, a negative regulator of muscle mass, and to assess whether changes in myostatin are associated with alterations in the systemic and local IGF system; (2) to determine the mechanism by which TNFalpha alters IGF and myostatin mRNA in cultured myocytes by assessing gene transcription and stability as well as elucidating the signaling pathways responsible for the changes in IGF and myostatin; (3) to determine the mechanism by which sepsis impairs in vivo growth hormone signaling in skeletal muscle by measuring activation of the JAK/STAT pathway and modulation of the suppressors of cytokine signaling (SOCS) protein family; (4) to determine whether the sepsis- induced change in IGF-1 influences muscle protein synthesis via an endocrine or autocrine/paracrine (muscle) mechanism, using transgenic mice with a liver-specific deletion of the igf 1 gene but normal IGF-1 expression in muscle; (5) to determine the mechanism by which the sepsis- induced increase in IGF binding protein-1 impairs muscle protein synthesis; and (6) to determine whether sepsis alters nontraditional components of the IGF system, such as the number of insulin.IGF-1 hybrid receptors and the expression of IGF binding protein-related protein-1, which can potentially regulate muscle protein synthesis by modulating insulin action. This proposed research is unique because it integrates both in vivo and in vitro studies designed to elucidate the cellular basis for the changes in IGF-1 during sepsis, and also because it directly assesses the interaction between TNF, the IGF system, and known regulators of protein synthesis. The data obtained will provide a more complete understanding of the factors influencing the IGF system and cellular metabolism, which is needed to realize the full potential and to avoid possible pitfalls of anabolic agents used in the management of the critically ill patient.

Grant: 2R01GM038436-14
Program Director: SCHWAB, JOHN M.
Principal Investigator: ROUSH, WILLIAM R
Title: ACYCLIC DIASTEREOSELECTION: METHODOLOGY AND SYNTHESIS
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 1988/02/01-2004/01/31

We propose to continue our research in the area of acyclic diastereoselective synthesis, with emphasis on novel allylmetalation reactions and fragment assembly aldol reactions which will be developed in the context of the total synthesis of structurally complex, biologically active natural products. Specific goals for the next grant period are: (1) Completion of a Total Synthesis of Tedanolide. The key step of the proposed synthesis was successfully modeled in the previous grant period, thus defining the strategy that will be pursued for completion of the total synthesis. (2) Allylation of Oxonium Ions; Synthesis of the Pyran Nucleus of Scytophycin C. A new strategy for the synthesis of the 2,6-trans dihydropyran unit of scytophycin C will be developed based on the reactions of allylsilanes and oxonium ions followed by ring closing metathesis. (3) Completion of a Total Synthesis of Scytophycin C. The total synthesis of scytophycin C will be completed. The key fragment assembly aldol step was successfully modeled in the previous grant period. (4) Total Synthesis of Spongistatin 1. A synthesis of the E-F bis-pyran unit was completed in the preceding grant period. Studies in the coming grant period will focus on the bifunctional gamma-silylallylborane for the convergent coupling of two aldehydes to generate 1,5-anti-pent-2(E)-1,5- diols, which will serve as precursors to the A-B and C-D spiroketals. A strategy for the stereocontrolled synthesis of the C-D spiroketal via inversion of the C(19) stereocenter also will be developed. (5) Total Synthesis of Apoptolidin. Fragment assembly aldol reactions of alpha-alkoxy ketones will be developed for the synthesis of the C(12)-C(28) segment of apoptolidin. (6) Total Synthesis of Amphidinol 3. Bifunctional allylboranes will be developed for the convergent coupling of two aldehydes to generate 1,5-syn-pent-2(Z)-1,5-diols, which will serve as key intermediates for the synthesis of the tetrahydropyran units of amphidinol 3. This methodology also provides an alternative strategy for synthesis of the scytophycin C dihydropyran nucleus.

Grant: 2R01GM039781-12
Program Director: FLICKER, PAULA F.
Principal Investigator: BOCIAN, DAVID F PHD CHEMISTRY:PHYSICA
Title: VIBRATIONAL STUDIES OF ENERGY TRANSDUCING PROTEINS
Institution: UNIVERSITY OF CALIFORNIA RIVERSIDE RIVERSIDE, CA
Project Period: 1988/07/01-2004/07/31

The specific aims of the proposed research are to characterize the structural, vibronic, and electronic properties of the functionally important cofactors in reaction center (RC) proteins. The principal investigative tool is resonance Raman (RR) spectroscopy. The first major objective is to characterize the properties of the bacteriochlorophyll/bacteriopheophytin (BCh1/BPh) in genetically modified bacterial RCs that exhibit unusual electron-transfer properties. The genetic modifications include replacements near the primary electron donor (P), both accessory BChls, and the BPh on the photophysically, active L branch of the protein (which is the primary electron acceptor). The studies focus on three general classes of genetically modified RCs: (1) Mutants in which the hydrogen bonding interactions and/or the electric fields in the vicinity of BCh1L, BCh1M, and BPhL are altered by addition/deletion of amino acid residues near ring V of the BCh1/BPh macrocycle. A particular focus of these studies concerns the effects of placing (potentially) charged residues near the photoactive cofactors. (2) Double (and higher order) mutants which incorporate the replacements characteristic of class 1 into a background in which BPhL is replaced with a BCh1 molecule (beta-type RCs). (3) Mutants in which the histidine axial ligands to the BChls of P are replaced by non-ligating glycine residues (cavity mutants). In all cases the RR studies will be conducted on RCs whose detailed electron-transfer kinetics have been elucidated via time-resolved optical experiments. The second major objective is to conduct RR studies aimed at refining the structure of oxygen-evolving complex in photosystem (PS) II RCs. The particular target of these studies is the manganese cluster, which directly mediates the water-splitting/oxygen-evolution reaction. The focus will be the low-frequency region of the spectrum where manganese-ligand vibrations are expected to occur. Toward this end, RR data will be acquired for PSII in which isotopic labels (^2H , ^{18}O , ^{15}N , ^{37}Cl -/ ^{37}Cl -) have been incorporated and/or essential ions such as Ca^{+2} and Cl^- have been exchanged (for example, Sr^{+2} for Ca^{+2} or Br^- for Cl^-). The long-term objective of the studies on bacterial and PSII RCs is to determine how the physical properties of the cofactors (structure, conformation, electron-density distribution) govern and/or reflect their functional characteristics (electron transfer/charge separation across the biological membrane; water splitting/oxygen evolution).

Grant: 2R01GM042686-10A1
Program Director: SOMERS, SCOTT D.
Principal Investigator: WINN, ROBERT K
Title: LPS INDUCED ENDOTHELIAL CELL ACTIVATION AND APOPTOSIS
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 1990/04/01-2004/05/31

Septic shock is a potentially lethal consequence of gram negative and positive bacterial infection and is a significant complication in victims of traumatic injury. There are multiple bacterial products implicated as pathogenic molecules including bacterial lipoproteins, lipopolysaccharide, (LPS), lipoteichoic acid, peptidoglycans, cell wall products, etc. Sepsis in experimental animals was shown to activate the intrinsic cell "suicide" program leading to apoptosis in multiple cell types. Insights into the molecular basis of cellular activation/apoptosis in response to sepsis are under intense investigation in the hope of finding new approaches to therapy. Signaling by bacterial products occurs through the recently described Toll-like receptors (TLR) on the surface of cells. Intracellular pathways leading to NFkappaB activation proceed along similar pathways for TLR-2 and TLR-4 (the two receptors shown to respond to bacterial products). However, apoptosis pathways have received less attention. The investigators will examine sepsis-induced apoptosis and a novel activation pathway in vitro as well as the effect of gene alterations that lead to decreased apoptosis in monocytes, lymphocytes and endothelial cells in vivo. They have recently shown that the apoptotic pathway following stimulation with LPS proceeds through FADD dependent signaling and that blockade of NFkappaB does not sensitize endothelial cells to death. These observations lead to questions regarding the death pathway and intrinsic cyto-protective pathways. Since considerable apoptosis occurs in LPS resistant (TLR-4 deficient) mice during sepsis, we also speculate that TLR-2 provides both activation signals and death signals in TLR-4 deficient mice. Also, they have observed two pathways leading to endothelial cell activation. First, mice lacking functional Fas (lpr) or FasL (gld) have reduced responses to LPS, and they postulate that the Fas-FasL system is pro-inflammatory. Second, a specific caspase-8 inhibitor reduces LPS-induced VCAM-1 expression. They postulate that this protease also signals for endothelial cell activation. The specific aims of this project are: 1) To determine the role of MyD88, Il-1 receptor- associated kinase and TNF receptor- associated factor-6 in sepsis-induced apoptosis of monocytes/macrophages and endothelial cells; 2) To determine the contribution of Fas-FasL and other molecules in that apoptosis pathway in LPS-induced inflammatory response of endothelial cells; and 3) To examine the effects of apoptotic gene alterations in monocytes, lymphocytes or endothelial in survival following induction of sepsis in mice.

Grant: 2R01GM044191-10A1
Program Director: FLICKER, PAULA F.
Principal Investigator: POCHAPSKY, THOMAS C
Title: STRUCTURE AND DYNAMICS OF METAL-CONTAINING PROTEINS
Institution: BRANDEIS UNIVERSITY WALTHAM, MA
Project Period: 1990/04/01-2003/12/31

This project represents a continuing effort to determine the role of structure and dynamics in determining function of metalloproteins. In particular, mechanisms of molecular recognition and interprotein electron transfer in the camphor hydroxylase pathway from *Pseudomonas putida* are the targets of investigation. This system consists of three proteins, the heme-containing cytochrome P-450/cam (CYP101), the Fe₂S₂ ferredoxin putidaredoxin (Pdx) and the NADH-dependent flavoprotein putidaredoxin reductase (PdR). Cyp101 catalyzes the 5-exo-hydroxylation of camphor by molecular oxygen, a reaction that requires two electrons supplied to the cytochrome sequentially by Pdx. Pdx is also required as an effector for substrate turnover. The electrons are supplied to Pdx by PdR by oxidation of PdR-bound NADH. The camphor hydroxylase system is a good model for human P450 enzymes involved in steroid hormone biosynthesis and processing of xenobiotics, drug metabolism and carcinogenesis. Improved methods are sought for determining structure and dynamics of paramagnetic proteins such as Pdx using selective isotope labeling and multinuclear NMR methods. A model for Pdx CYP101 interactions has been developed and will be tested using protein engineering (mutagenesis) and NMR characterization of the solution complexes. Advances in NMR methodology, along with improved expression techniques in deuterated media, have allowed the first high resolution multi-dimensional NMR spectra of a uniformly ²H(¹³C), ¹⁵N- labeled CYP101 to be presented. Structure-activity relationships of a novel metalloenzyme, E2, are also under investigation. E2 is involved in the methionine salvage pathway, and produces different products from the same substrate depending on the metal bound in the E2 active site. We have determined the global fold of E2 by NMR and are investigating the differences in substrate binding between the different metal-containing isoforms of the enzyme. The methionine salvage pathway is important for feedback inhibition of polyamine biosynthesis, which are associated with tissue pathology in mammals.

Grant: 2R01GM045844-10
Program Director: ANDERSON, JAMES J.
Principal Investigator: KILEY, PATRICIA J PHD
Title: REGULATION OF GENE EXPRESSION BY OXYGEN
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 1991/04/01-2004/03/31

DESCRIPTION: (Applicant's abstract) Determining how cells sense and adapt to fluctuating O₂ levels in their environment is a fundamental problem in biology. For many prokaryotic and eucaryotic organism, the sensing of oxygen levels is essential to ensure an adequate supply of energy as well as to avoid the toxic effects of oxygen. In recent years, it has been shown that many members of the FNR family of transcription factors that play a key role in the anaerobic lifestyle of a wide group of prokaryotes, function in oxygen sensing. Thus by deciphering how FNR activity responds to oxygen availability, we will obtain fundamental information on a process that is critical to the growth and survival of facultative microbes. In addition, our studies should provide key insights into some general properties of oxygen sensing that can be applied to both eucaryotic and prokaryotic cells. In the best-studies case of *Escherichia coli*, FNR contains a [4Fe-4S]₂ cluster that is required for dimerization and site-specific DNA binding. This [4Fe-4S]₂ cluster is oxygen sensitive and its conversion to a [2Fe-2S]₂ decreases dimerization and DNA binding in vitro. To explain how FNR activity is regulated in vivo, we propose that FNR is largely active under anaerobic conditions because the [4Fe-4S]₂ cluster is stable whereas, in the presence of oxygen, we propose that FNR is largely inactive due to its conversion to a [2Fe-2S]₂ intermediate or possibly an apo-FNR form that lacks a cluster. To determine whether the [4Fe-4S]₂ cluster conversion is sufficient to explain how FNR is inactivated under aerobic conditions in vivo, we will define the pathway of FNR inactivation in vivo. To determine how the presence of the [4Fe-4S]₂ cluster increases FNR activity, we will test the hypothesis that the [4Fe-4S]₂ cluster is required to achieve a conformation that is competent for dimerization. To further define the role of FNR as a global regulator of transcription we will characterize the regions of FNR involved in transcription activation. Our studies should provide insights into conserved regulatory strategies for sensing changes in oxygen tension by a wide variety of prokaryotes including several pathogenic organisms.

Grant: 2R01GM045898-10
Program Director: ANDERSON, JAMES J.
Principal Investigator: ZUBER, PETER A
Title: SRF OPERON--REGULATION AND ROLE IN GENETIC COMPETENCE
Institution: OREGON GRADUATE INSTITUTE SCIENCE & BEAVERTON, OR
TECH
Project Period: 1992/02/01-2001/08/31

DESCRIPTION (adapted from the investigator's abstract): Bacteria possess regulatory networks that sense environmental and metabolic conditions, and respond by generating and transducing signals that affect gene expression. Starvation and high cell density influence the production of virulence factors such as toxins, antibiotics, and degradative enzymes through regulatory networks. They also induce complex cell differentiation processes that give rise to resistant cell types or competent cells that can acquire exogenous DNA. In *Bacillus subtilis*, the *srf* operon resides within a regulatory network that governs processes induced by nutrient depletion and high cell density. *srf* encodes surfactin synthetase, an antibiotic biosynthesis operon, and ComS, a regulatory peptide that controls competence development. The major goal of the project is to understand how *srf* and *comS* are regulated and how ComS stimulates competence development. *srf* is under the control of two converging regulatory pathways. One mediates quorum-sensing control and involves the two-component regulators ComP and ComA; phosphorylated ComA activates *srf* transcription. The other pathway, involving the Phr extracellular peptide and the SpoOK oligopeptide permease, is activated by starvation and high cell density; the Phr peptide, imported via SpoOK, inhibits the Rap phosphatase that converts ComA to an inactive form, allowing interplay between the two pathways. Activation of *phr* expression requires the SigmaH form of RNA polymerase, the activity of which is induced by starvation and requires ClpX, an ATP-dependent chaperone. The role of ClpX in the activation of E-SigmaH will be determined by purification and reconstitution of RNA polymerase in vitro for transcription reactions containing purified ClpX proteins. Mutations which suppress the phenotype of a *clpX* mutant will be characterized to identify factors influencing ClpX-dependent activation of E-SigmaH. Other functions of ClpX in the activation of *srf* transcription will be identified by testing the effects of *clpX* *comP* and *clpX* *spoOK* double mutants on *srf* expression. ComS is required to release ComK, the transcriptional activator of competence gene expression, from the competence inhibitory proteins MecA and ClpC. The ComS-dependent release is thought to rescue ComK from regulated proteolysis. A collection of ComS point mutations will be analyzed to determine the function of ComS in the activation of competence gene expression. These studies will further understanding of the functional links between stress-induced proteins and the regulation of prokaryotic cellular differentiation.

Grant: 2R01GM047112-28
Program Director: IKEDA, RICHARD A.
Principal Investigator: SWITZER, ROBERT L PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: NOVEL CONTROL MECHANISMS IN ENDOSPORE FORMATION
Institution: UNIVERSITY OF ILLINOIS URBANA- CHAMPAIGN, IL
CHAMPAIGN
Project Period: 1991/05/01-2004/03/31

Abstract Text Not Available

Grant: 2R01GM047444-09
Program Director: ZATZ, MARION M.
Principal Investigator: KAPLAN, HEIDI B PHD MICROBIOLOGY, OTS
Title: TRANSDUCTION OF THE A SIGNAL IN MYXOCOCCUS DEVELOPMENT
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX
HOUSTON
Project Period: 1992/05/01-2004/04/30

Myxococcus xanthus represents an excellent model system to address fundamental questions of how cell-cell signaling pathways control multicellular development. These questions are relevant to all normal embryonic and adult cells that transduce signals to coordinate processes such as growth and differentiation, as well as to cells that are defective in signaling networks, such as cancer cells. Progression through early multicellular development requires that *M. xanthus* cells sense and respond to a high cell density and nutrient limitation. Two sensitive sensing networks monitor these extracellular signals and converge at a critical checkpoint early in *M. xanthus* development. This check -point can be monitored by expression of a specific gene, *spi*. The long-term goals of this research are to determine: i) How do the cells sense and transduce the cell-density signal? ii) How are the cell-density- and nutrient-sensing pathways integrated? iii) What is the connection between the change in gene expression and the complex behavioral response of multicellular fruiting body formation? The identification and characterization of three critical regulators of *spi* expression, SasS, SasR, and SasN has begun to address these questions. Using classical and molecular genetics combined with protein biochemistry, they have generated a hypothesis for the mechanism by which these proteins integrate both the cell-density signal (extracellular A signal) and the starvation signal, creating the circuitry that controls the developmental expression of the responsive *spi* gene. To test this hypothesis, they plan to: i) analyze the SasS/SasR/SasN-dependent integration of cell density and starvation signals during early *M. xanthus* development, ii) generate an in vitro transcription system to test the activity of the SasS/SasR pathway, iii) analyze the stimulation of the SasS/SasR pathway by alterations in cell-surface integrity, and iv) investigate SasS/SasR-independent A signal transduction pathways. Their ultimate goal is to reconstitute the complete signaling pathway as proof of its function.

Grant: 2R01GM047856-05A2

Program Director: SHAPIRO, BERT I.

Principal Investigator: KUNG, CHING PHD

GENETICS:BIOCHEMICAL/M

LECULAR

Title: STRUCTURE FUNCTION OF BACTERIAL CHANNELS

Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI

Project Period: 1994/05/01-2004/02/29

Abstract Text Not Available

Grant: 2R01GM051986-06
Program Director: GREENBERG, JUDITH H.
Principal Investigator: BRUN, YVES V
Title: GLOBAL CONTROL OF DIFFERENTIATION IN CAULOBACTER
Institution: INDIANA UNIVERSITY BLOOMINGTON BLOOMINGTON, IN
Project Period: 1995/01/01-2003/12/31

DESCRIPTION: Cell division in the bacterium *Caulobacter crescentus* produces two different cell types: a motile swarmer cell and a sessile stalked cell. Only the stalked cell is capable of DNA replication and cell division. The long term goal of this project is to understand how cell division and cell differentiation are regulated and coordinated in *Caulobacter*. *Caulobacter* is particularly well suited for these studies because it undergoes a relatively simple developmental program with distinct morphologies, and because cell populations can be synchronized easily. The first objective is to define the cell cycle and developmental controls of cell division initiation through an understanding of the factors that control FtsZ. As part of this objective, Dr. Brun plans to explore the mechanism of cell type-specific proteolysis of FtsZ, determine the developmental and temporal control of FtsZ localization, and identify proteins that interact with FtsZ. The second objective is to characterize the regulatory sequences and trans-acting factors responsible for regulation of *ftsQA* and determine how the transcription of *ftsQA* is coupled to DNA replication. The final objective is to identify the sigma-54-dependent genes that are required to link pole development to cell division and to define the function of *stpA*, which appears to be required for proper localization of the stalk and holdfast.

Grant: 2R01GM052051-06A1
Program Director: TOMPKINS, LAURIE
Principal Investigator: OSUNA, ROBERT PHD BIOLOGY
Title: REGULATION AND FUNCTION OF FIS IN E COLI
Institution: STATE UNIVERSITY OF NEW YORK AT ALBANY, NY
ALBANY
Project Period: 1995/01/01-2004/06/30

DESCRIPTION (adapted from the investigator's abstract): Fis is a small DNA binding protein that plays an important role in a number of cellular processes in E. coli and other enterics, including site-specific recombination systems (e.g. Hin and Gin) and transcription initiation (e.g. rRNA and some tRNA promoters). Its regulation pattern is interesting, with synthesis dramatically increasing from undetectable to 25,000-50,000 molecules/cell following upshift. The goals of this grant are (1) to understand the mechanism of regulation of fis transcription, focusing primarily on the role of initiating nucleotide concentrations (CTP), IHF, and Fis itself on the activity of the fis promoter; (2) to determine what other protein products are controlled by Fis; and (3) to understand the determinants of DNA recognition by Fis.

Grant: 2R01GM052465-05A1
Program Director: ANDERSON, JAMES J.
Principal Investigator: FARRAND, STEPHEN K
Title: CELL-CELL SIGNALING IN MICROBE-HOST INTERACTIONS
Institution: UNIVERSITY OF ILLINOIS URBANA- CHAMPAIGN, IL
CHAMPAIGN
Project Period: 1995/05/01-2003/12/31

Interactions between microbes and their hosts, including man, depend upon specific communication systems. Thus, bacteria, including pathogens, perceive their hosts by sensing chemical signals, and respond appropriately. Understanding these signaling pathways, and how the microbe and its host respond, could lead to strategies for preventing pathogenesis or fostering beneficial relationships. *Agrobacterium tumefaciens* which interacts with plants, provides an excellent model. As a part of this interaction, the bacterium responds to a plant signal by eliciting a second signal perceived by the entire bacterial population. This second, quorum-sensing signal controls transfer of the Ti plasmid, a virulence element, to other bacteria. The long term goal is to understand this hierarchical signaling process including how the quorum sensing signal, an acyl-homoserine lactone called AAI regulates the activity of TraR, the quorum-sensing transcriptional activator required for expression of the Ti plasmid conjugation system. There are three goals for the project period. First, primer extension, and DNA footprinting will be used to determine how AccR, the plant signal-responsive repressor regulates a divergent promoter system responsible for expression of TraR. This region contains several regulated promoters; one controlled by phosphorus availability and two or more controlled by AccR. Deletions will be used to identify cis acting elements responsible for these activities. Second, using wild-type and mutant alleles, novel genetic screens as well as in vitro assays with purified protein will be employed to examine the multimeric nature of TraR, the role played by AAI in converting TraR into an active activator, for forming multimers, for binding promoters, and for activating transcription. The nature of the interaction between unactivated TraR and inner membrane will be determined, as well as the role of AAI in converting TraR into a cytoplasmic protein. The role of *Agrobacterium* RpoA in gene activation by TraR will be assessed. If they interact regions of the two proteins involved in this interaction will be identified. Mutants of TraR affected in affinity for AAI will be isolated and used to probe the mechanism by which AAI activates the protein. Third, genetic screens, as well as in vitro interaction assays will be used to determine how the antiactivator, TraM, interacts with TraR. Using physiological and biochemical assays the role of TraM in targeting TraR for proteolytic degradation will be determined. The pathways for this proteolysis will be identified by isolating and characterizing *Agrobacterium* mutants that are unable to degrade TraR in the presence and absence of AAI.

Grant: 2R01GM052725-05
Program Director: ANDERSON, RICHARD A.
Principal Investigator: COX, MICHAEL M
Title: BACTERIAL PROTEINS INVOLVED IN DNA REPAIR
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 1996/03/01-2004/06/30

DESCRIPTION (adapted from the investigator's abstract): The primary function of homologous genetic recombination in bacteria is the nonmutagenic repair of arrested replication forks under normal growth conditions. Virtually every replication fork originating at *oriC* encounters DNA damage at some point, and must undergo recombinational DNA repair. This process represents perhaps the most complex and certainly the least understood of the major pathways for DNA repair. The goal of the work supported by GM52725 is a complete understanding of these critical repair pathways, where the replication and recombination systems are closely integrated. Five specific aims are proposed. First, fundamental biochemistry of several proteins involved in recombinational DNA repair will be explored, focusing on the RecF, RecO, RecR, and RecG proteins. Second, the interaction of a variety of recombination proteins with RecA protein will be explored. This effort will focus on the RecG protein, along with the RuvA and RuvB proteins. In the third aim, DNA substrates will be constructed to mimic at least one of the proposed DNA structures that may occur at arrested replication forks. They will then determine how these DNA structures are processed by recombination enzymes, including the RuvAB complex, the RecG protein, and the RecA proteins. To further elucidate the fate of replication forks when they encounter DNA damage, a fourth aim is to examine the biochemistry of replication fork arrest systematically, using DNA substrate with different types of DNA damage embedded site-specifically. The last aim is to examine the mutagenic replicative bypass of DNA lesions by DNA polymerase V *in vitro*. These experiments are designed to reconstitute parts of the proposed pathways for replication fork reactivation, to further the ultimate goal of a complete reconstitution with purified enzymes.

Grant: 2R01GM052840-06
Program Director: CHIN, JEAN
Principal Investigator: BABITZKE, PAUL L
Title: MECHANISM OF TRP GENE REGULATION BY TRAP-RNA RECOGNITION
Institution: PENNSYLVANIA STATE UNIVERSITY-UNIV UNIVERSITY PARK, PA
PARK
Project Period: 1995/08/01-2004/07/31

Post-transcriptional regulatory mechanisms play a role in gene expression in probably all organisms. TRAP of *Bacillus subtilis* regulates expression of the *trpEDCFBA* operon by transcription attenuation and translational control mechanisms. When activated by tryptophan, TRAP binds to a 5' stem-loop (5'SL) and 11 (G/U)AG repeats in the nascent *trp* leader transcript, thereby promoting transcription termination before RNA polymerase can reach the *trp* structural genes (attenuation). TRAP binding also promotes formation of an RNA structure that inhibits *trpE* translation. These mechanisms will be further analyzed to gain a better understanding of *B. subtilis* *trp* operon regulation and the diversity of post-transcriptional regulatory mechanisms. Since several human disorders are caused by sequestration of triplet repeat RNA-binding proteins, combined with the findings that expression of HIV and several oncogenes is regulated by attenuation, results from these studies will indirectly contribute to improving human health. The role that the 5'SL plays in *trp* operon expression will be investigated. The amino acid residues of TRAP that interact with the 5'SL, as well as the 5'SL nucleotides that interact with TRAP will be determined by TRAP-5'SL RNA binding studies. The possibility that the 5'SL increases the rate of TRAP-*trp* leader RNA association will also be examined by performing binding assays. In addition, *in vitro* and *in vivo* experiments will be performed to determine if the 5'SL serves as an RNA polymerase pause signal and/or an mRNA instability determinant. The mechanism of translational control mediated by TRAP-dependent formation of the *trpE* Shine-Delgarno (SD) blocking hairpin will also be examined. *In vivo* expression studies will be carried out to determine if the 5'SL participates in this mechanism. *In vivo* expression, *in vitro* translation, and mRNA half-life experiments will also be performed to determine if formation of the *trpE* SD blocking hairpin regulates expression of the downstream genes via translational coupling, transcriptional polarity and/or decreasing mRNA stability. In addition, experiments will be carried out to determine if translational control requires a higher tryptophan concentration than is required for transcription attenuation. Finally, the possible role of an RNA pseudoknot in regulating translation and/or mRNA stability of the *trp* operon will be determined by several genetic and biochemical approaches.

Grant: 2R01GM053146-05A1
Program Director: RHOADES, MARCUS M.
Principal Investigator: COOPERMAN, BARRY S PHD CHEMISTRY:ORGANIC
SYNTHESIS
Title: NUCLEIC ACID PROBES OF RIBOSOMAL STRUCTURE AND FUNCTION
Institution: UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA
Project Period: 1995/08/01-2004/03/31

The ribosome is the unique site of protein biosynthesis in all cells, and as such a detailed understanding of its structure and function is of fundamental importance to the more general understanding of cellular function at the molecular level. Aside from its intrinsic importance to the basic comprehension of life processes, better understanding of ribosomal function could have important therapeutic consequences. Many antibiotics in current clinical use, such as tetracycline, erythromycin and other macrolides, neomycin and other aminoglycosides, and chloramphenicol target ribosomes as their sites of action. Interest in these ribosomal antibiotics has been growing as bacterial resistance to beta-lactams and quinolones has become more widespread. Several drug companies are now devoting considerable resources toward synthesizing analogues and derivatives of ribosomal antibiotics that overcome bacterial resistance. Better understanding of ribosomal structure and function will be especially important for antibiotics, such as macrolides, where resistance is based on changes in ribosome structure. Our studies will be carried out on the *E. coli* ribosome, which is by far the best characterized by the studies of many groups, including our own. However, given the considerable conservation of ribosome structure throughout evolution the results we obtain should also be useful for understanding ribosomes from other organisms. The overall goal of this proposal is to describe conformational changes that the ribosome undergoes during specific steps of its functional cycle and how mutations and antibiotic binding affect these changes. We propose to do this by forming defined photocrosslinks from rRNA sites within the ribosome that have been targeted on the basis of their importance for ribosome structure and function, taking advantage of the intrinsic ability of the photocrosslinking process to sample all conformations in solution. Such crosslinks will be formed in different functional states, in wild-type and mutant ribosomes, and in the presence and absence of antibiotics. The structural constraints represented by such crosslinks, along with constraints generated by other approaches, will be used to model structures of the ribosome in specific functional states, using crystal structures of 70S ribosomes and 30S and 50S subunits as initial structures. As our major approach we will continue and refine the use of radioactive, photolabile derivatives of oligonucleotides having sequences complementary to rRNA sequences (PHONTs). Such probes bind to their targeted sequences in intact ribosomal subunits, and, on photolysis, incorporate into neighboring ribosomal components that can subsequently be identified. We also will develop a second approach based on site-specific introduction of photolability into intact rRNA (IPHOR - intact photolabile RNA) to obtain similar information for rRNA sites that are either inaccessible to PHONTs or where the use of PHONTs induces major conformational change.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2R01GM053269-06
Program Director: MARINO, PAMELA
Principal Investigator: IP, YICKTUNG T PHD
Title: MOLECULAR MECHANISMS OF DROSOPHILA IMMUNE RESPONSES
Institution: UNIV OF MASSACHUSETTS MED SCH WORCESTER, MA
WORCESTER
Project Period: 1995/05/01-2004/03/31

DESCRIPTION (verbatim from the application): The goal of the proposed research is to use molecular genetic methods to determine the basis for innate immunity in a model organism, *Drosophila melanogaster*. Upon infection, insects mount an immune response by mobilizing the blood cells and producing a spectrum of antimicrobial peptides to engulf and to lyse the invading microorganisms. This insect self-defense process is similar to the mammalian innate immune system. The innate immune system first recognizes common features of infectious agents, such as the Gram-negative bacteria membrane component lipopolysaccharide (LPS). This leads to signaling events in liver cells, endothelial cells, and macrophages to produce protective molecules and cytokines. These responses serve as the first line defense and help to stimulate B and T lymphocytes of the acquired immune system. Recent studies demonstrate that insect and mammalian innate immunity share a common evolutionary origin. In particular, a *Drosophila* regulatory factor, Dif, is related to mammalian NF-kappaB. Moreover, the *Drosophila* transmembrane protein Toll is essential for the activation of Dif, while the mammalian Toll-like receptors activate NF-kappaB and mediate LPS stimulated immune response. Recent results also suggest that *Drosophila* MAP kinases participate in insect immunity. MAP kinases are mediators of inflammatory response in mammals, further supporting the idea that mammalian and insect immunity employ many homologous molecules. This proposal aims at testing the hypothesis that the Toll signaling, the MAP kinase pathways, and possible novel molecules regulate different aspects of *Drosophila* immunity. The successful achievement of the proposed research should provide important insights into the evolutionarily conserved immune process in humans. This proposal includes three specific aims: (1) Analyze how Dif protein dimers specifically regulate a subset of immunity genes; (2) Identify novel Dif target genes and new genetic components in the self-defense process; (3) Study the roles of MAP kinase pathways in insect immunity.

Grant: 2R01GM053825-05
Program Director: EDMONDS, CHARLES G.
Principal Investigator: ARMSTRONG, DANIEL W
Title: CHIRAL RECOGNITION BY MACROCYCLIC GLYCOPEPTIDES
Institution: UNIVERSITY OF MISSOURI ROLLA ROLLA, MO
Project Period: 1996/03/05-2000/08/15

Macrocyclic antibiotics are the newest and fastest growing class of chiral selectors for enantiomeric separations. Enantiomeric separation and evaluation are now mandated by the Food and Drug Administration for all pharmaceutical compounds that can exist as racemic modifications. Approximately 75 percent of all pharmaceutical products are chiral. Currently the mechanism(s) by which macrocyclic antibiotics achieve chiral recognition and enantioselective separation is unknown. There are three basic aims/objectives to this proposed work. They are: (1) elucidate the chiral recognition/enantioselective separation mechanism specifically for the glycopeptide antibiotics. (2) Synthesize and evaluate a new type of very simple macrocyclic glycopeptide (note that all known macrocyclic glycopeptides are produced biologically), and (3) Evaluate two unique, anionic-boron containing macrocyclic antibiotics. This work should greatly expand our understanding of these important compounds as well as their utility in the enantiomeric separation of biologically important molecules.

Grant: 2R01GM053836-06
Program Director: SHAPIRO, BERT I.
Principal Investigator: KLEBBA, PHILLIP E
Title: LIGAND GATED TRANSPORT THROUGH FEPA
Institution: UNIVERSITY OF OKLAHOMA NORMAN NORMAN, OK
Project Period: 1995/09/30-2004/03/31

DESCRIPTION (Adapted from the Applicant's Abstract): Bacteria elaborate iron chelators that scavenge iron from the environment, including their human and animal hosts. Iron acquisition is a determinant of pathogenicity. One such iron chelate, the siderophore enterobactin, enters gram-negative bacteria through the FepA protein of the outer membrane. FepA is a ligand-gated porin, in that binding of ferric enterobactin triggers transport through its transmembrane pore. This high affinity multispecific, multicomponent, energy dependent transport process is a paradigm of prokaryotic membrane biochemistry. Based on the FepA crystal structure, the proposed research will use molecular biological, biochemical, and biophysical methods to investigate the mechanism of ferric enterobactin uptake. The experiments will address two stages of the transport event, binding and internalization. Dr. Klebba will study the specificity of the initial recognition event by binding experiments on both wild type FepA and site-directed mutants, containing alterations to residues in either the external loops of the top loops of the N-terminal globular domain. He will similarly characterize the ligand internalization reaction by mutagenesis of target residues that are conserved among other Gram-negative bacterial ligand-gated porins. Mutant proteins of interest, those with impaired ligand binding or ligand internalization phenotypes, will be crystallized and studied by X-ray diffraction. Finally, he will perform biophysical analyses of conformation changes that occur in FepA during ligand transport.

Grant: 2R01GM054033-05
Program Director: LEWIS, CATHERINE D.
Principal Investigator: WAKSMAN, GABRIEL PHD
Title: STRUCTURAL STUDIES OF DNA REPLICATION AND REPAIR
Institution: WASHINGTON UNIVERSITY ST LOUIS, MO
Project Period: 1996/05/01-2004/04/30

The molecular structures of proteins involved in two aspects of DNA replication and repair, DNA unwinding and DNA polymerization, will be studied utilizing the techniques of x-ray crystallography. DNA bound and free forms of the proteins, as well as site-directed mutants, will be investigated. During the last grant period, four protein systems involved in these processes were studied: the DNA polymerase I from *Thermus aquaticus*, *Escherichia coli* single strand DNA-binding protein SSB, and the *E. coli* dimeric DNA-helicases Rep and UvrD. These proteins will be studied further, while the structural studies of one additional protein system, the DNA polymerase delta holoenzyme from *Saccharomyces cerevisiae*, will be initiated. The Taq DNA polymerase is involved in DNA repair, while the Polymerase delta holoenzyme is the major replicative enzyme in eukaryotes. The Taq DNA polymerase is also an important biotechnological tool, used extensively in DNA sequencing and in the polymerase chain reaction. The crystal structures of nine different ligated states of an active N-terminal deletion of Taq polymerase corresponding to the Klenow fragment of *E. coli* of DNA polymerization have been determined. Additional complexes will be studied, as well as the full-length Taq polymerase which contains at its N-terminus a 5'-3' exonuclease activity. Studies of components of the polymerase delta holoenzyme will be initiated, which will shed light into the process of replicative DNA polymerization, which has been conserved from phage T4 to human. DNA unwinding is a fundamental process in DNA replication and repair. Several human diseases, including xeroderma pigmentosum and Cockayne's syndrome, involve defects in proteins that possess helicase activity. *E. coli* Rep and UvrD are involved in active unwinding of DNA, while *E. coli* SSB is involved in passive DNA helix destabilization. Rep and UvrD both function as dimers and are motor proteins which use the energy derived from ATP-binding and hydrolysis to cycle kinetically through conformational states. The structures of the Rep helicase bound to a single-stranded DNA, as well as the structure of the DNA binding domain of *E. coli* SSB were determined. Structures of UvrD, as well as of complexes of SSB with DNA, are in the process of being solved. Various functionally-relevant complexes of these proteins, kinetically trapped at various stages of unwinding, will be studied. Such structures will provide information on the mechanism of active unwinding by DNA-helicases and of passive unwinding by helix-destabilizing SSB proteins.

Grant: 2R01GM054060-04
Program Director: SOMERS, SCOTT D.
Principal Investigator: GOLENBOCK, DOUGLAS T
Title: PHAGOCYTE RECEPTORS FOR LIPID A
Institution: BOSTON MEDICAL CENTER BOSTON, MA
Project Period: 1999/01/01-2004/03/31

Gram-negative bacterial sepsis is a medical catastrophe, claiming the lives of 35-40 percent of affected patients. There has been little progress in improving the poor outcome of sepsis, due to our inadequate understanding of the basic pathophysiology of the syndrome. Sepsis results from a cascade of life-threatening host responses beginning with the interaction of bacterial lipopolysaccharide (LPS) and the LPS-receptor complex on effector cells. CD14 appears to be the LPS-binding component of this complex. The exact components of the complex, and the mechanism of signal transduction, are uncertain. Two Toll-like receptors (TLRs), TLR2 and TLR4, function as LPS signal transducers. Expression of TLR4, but not TLR2, is obligatory for sensitive responses to LPS. TLR2 functions as a pattern recognition molecule for bacterial products (including LPS) from multiple types of bacteria. LPS-receptor specificity resides in TLR4: expression of TLR4 defines a highly specific pharmacology to lipid A and its analogs. The central hypothesis of this proposal is that LPS is recognized by a multimeric receptor consisting of CD14 and of a complex of TLRs and accessory molecules. TLR4 is the predominant LPS receptor in most cells; TLR2 may be important for sCD14/LPS recognition. Upon ligand binding, TLRs multimerize and engage signaling molecules similar or identical to those used by the IL-1 receptor. LPS antagonists may function by preventing TLR multimerization. We propose three Aims to test this hypothesis. 1. To analyze available known TLRs (including TLRs 1, 3, 5, 6 and 7) and chimeric TLR constructs as potential LPS receptor components. To identify regions of TLR4 that are necessary for specific ligand recognition. To determine if epitope-tagged TLRs multimerize, and if multimerization is inhibited by LPS antagonists. 2. To use [32P]-lipid IVa to analyze lipid A binding to TLRs, especially TLR4. 3. To identify new genes in the LPS signal transduction pathway using somatic cell mutagenesis and complementation analysis approaches. The discovery of Toll-like receptors as mediators of signal transduction for both Gram-positive and Gram-negative bacteria suggests that there are common signaling elements that can be discovered and targeted for the rational design of novel therapeutic agents for sepsis, and thus reduce the appalling morbidity and mortality associated with this disease.

Grant: 2R01GM054063-38A1
Program Director: SCHWAB, JOHN M.
Principal Investigator: RINEHART, KENNETH L PHD
CHEMISTRY:CHEMISTRY-
UNSPEC
Title: STRUCTURES OF ANTIBIOTICS AND RELATED COMPOUNDS
Institution: UNIVERSITY OF ILLINOIS URBANA- CHAMPAIGN, IL
CHAMPAIGN
Project Period: 1977/05/01-2004/07/31

DESCRIPTION (verbatim from applicant's abstract): The present research proposal is designed to test the hypothesis that marine organism-derived natural products can provide clinically useful pharmaceutical agents. Specifically, antitumor agents will be sought, and to a lesser degree antimicrobial, antiviral, and immunosuppressive agents. These represent multiple goals of which the antitumor target is the most readily accessible and is now in sight. Research will focus on ecteinascidin 743 (ET 743) and dehydrodidemnin B (Aplidine). These two compounds from our laboratory are now in clinical trial in Europe and North America as anticancer agents. ET 743, from the tunicate (sea squirt) Ecteinascidia turbinata, currently in Phase II clinical trials, is present in only 1 g/ton of the tunicate and efforts will be made to increase the quantity available for human testing by defining the biosynthetic pathway, including precursors and intermediates. Attempts will be made to establish whether a symbiotic microorganism may actually produce ET 743. More active derivatives and analogs of ET743 will be sought and developed. Oxidative degradation will be studied to characterize the metabolites and perhaps to slow drug degradation in patients. Aplidine (dehydrodidemnin B) is currently in Phase I trials and may be regarded as a second-generation analog of didemnin B (DB). The latter reached Phase II trials and showed promise in treating non-Hodgkins lymphoma but was dropped due to cardiac toxicity. By contrast Aplidine is up to 30 times as active as DB and lacks its cardiotoxicity. Biosynthesis of Aplidine and DB will be studied in Spain and the Caribbean, respectively, but is complicated by its symbiotic relationship with the cyanobacterium Synechocystis trididemniii, which will be studied separately. Ring opened analogs of Aplidine and DB will be prepared to see whether they represent biosynthetic intermediates or degradation products of the cyclic depsipeptide. Amide analogs of the Hip and Thr units will also be prepared, to stabilize the cyclic depsipeptides and hinder in vivo hydrolysis. A cyanobacterial product, a mixture of oscillacidins A and B from an Oscillatoria species, appears to be toxic to L1210 leukemia cells and is being investigated for its unique structure, different from the hepatotoxins microcystins and nodularin, which are cyclic heptapeptides and pentapeptides containing the novel acid Adda.

Grant: 2R01GM054226-05
Program Director: WOLFE, PAUL B.
Principal Investigator: TSE-DINH, YUK-CHING PHD
BIOCHEMISTRY:BIOCHEM
RY-OTHER
Title: CONTROL OF DNA TOPOLOGY
Institution: NEW YORK MEDICAL COLLEGE VALHALLA, NY
Project Period: 1996/04/01-2004/03/31

Due to the recent emergence of pathogenic bacteria resistant to all antibiotics currently used, there is an urgent need to develop new antibiotics against novel targets. Bacterial topoisomerase I is a promising new target for antibacterial therapy with lead compounds having MIC's of 4.0 µg against *Staphylococcus aureus*. *E. coli* topoisomerase I is the best studied example of bacterial topoisomerase I and share extensive homology with topoisomerase I from both gram-positive and gram-negative bacteria. Topoisomerase I targeting drugs that inhibit DNA religation would lead to cell killing in a mechanism similar to those of many drugs targeting bacterial DNA gyrase and human topoisomerases. Loss of topoisomerase I function may also affect the ability of the bacteria to respond to environmental challenges encountered in pathogenesis. The long term goals of this project include the elucidation of the mechanism, regulation and physiological roles of *E. coli* topoisomerase I, which would greatly aid the development of novel bacterial agents targeting this class of enzyme. The aims for this proposal include: 1. Structure-function analysis by different mutagenesis approaches to identify residues required for the individual steps of catalysis by *E. coli* topoisomerase I 2. Limited proteolysis and chemical cleavage of topoisomerase I in the absence and presence of DNA to identify sites of cleavage altered due to either enzyme conformational change or protection by DNA substrate. 3. Test of peptide sequences identified by panning as potential inhibitor of topoisomerase I 4. Study of the molecular basis of topoisomerase I involvement in bacterial adaptation to environmental challenges for survival.

Grant: 2R01GM054365-18
Program Director: DEATHERAGE, JAMES F.
Principal Investigator: ORDAL, GEORGE W
Title: CHEMOTACTIC SENORY TRANSDUCTION IN BACILLUS SUBTILIS
Institution: UNIVERSITY OF ILLINOIS URBANA- CHAMPAIGN, IL
CHAMPAIGN
Project Period: 1983/08/01-2004/05/31

DESCRIPTION: (Adapted from the Investigator's Abstract) One major outcome of the study of chemotaxis during the past decade is that the well-studied enteric model may not be representative of archaea and eubacteria ("bacteria"). Taxis in the archeon *Halobacterium salinarum* bears some remarkable similarities to that in *Bacillus subtilis*, a finding that suggests that the ancestral mechanism might have been closer to these mechanisms, rather than to the simplified mechanism in the enteric bacteria. For this reason, this work might establish a new paradigm for chemotaxis, which may be followed by the great sweep of eubacteria and archaea. It is becoming increasingly clear that complexes of receptors with other proteins control output, not just in chemotaxis but in many systems. However, bacterial chemotaxis is an especially good model system as virtually any combination of mutations, including the charge at each site of receptor methylation, can be used to ask specific questions and the effects studied in vivo and in vitro. In this proposal we are focusing on the interactions among chemotaxis proteins, including the receptors and the excitatory CheA kinase, in order to define the structure/function relationships. Our recent experiments have led to the hypothesis that CheY-P feeds back on the receptor to inhibit CheA during adaptation and may even be hydrolyzed at the receptor, unknown features in *Escherichia coli* chemotaxis. Such an interaction may occur in *H. salinarum* and may represent the ancestral adaptation mechanism. It appears that the receptors control CheY-P levels through differential regulation of the state of methylation/amidation of each of the sites of receptor methylation. Some proteins have no counterparts in *E. coli* -- CheC, CheD, and CheV. It appears that CheC/CheD, present also in *H. salinarum*, controls the processes that regulate CheY-P levels. Phosphorylation of CheV helps bring about adaptation. Exploring these subjects may revolutionize our understanding of how chemotaxis works in *B. subtilis* and perhaps in many bacteria. The goal is to elucidate these specific hypotheses or findings. Where the receptors and CheY-P interact and the consequences of the interaction in vivo and in vitro will be determined. The effect of CheV on this interaction will be explored. How degree of methylation at each site in the asparagine receptor, McpB, affects CheA and the CheY-P interaction as well as behavior will be quantitated. The purpose and location of interactions of CheC and CheD with each other and with McpB and CheA (in the case of CheC) will be elucidated. The (fast) kinetics of CheA autophosphorylation and phosphotransfer to CheY, CheB, and CheV and their purposes will be studied. The effect of receptor interaction on these kinetics will be explored.

Grant: 2R01GM054395-18
Program Director: GREENBERG, JUDITH H.
Principal Investigator: MORAN, CHARLES P PHD GENETICS:GENETICS
OTHER
Title: RNA POLYMERASE AND BACTERIAL DIFFERENTIATION
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 1996/06/01-2004/05/31

As the bacterium *Bacillus subtilis* differentiates from the vegetative form into a dormant endospore, complex morphological changes occur that require the expression of many genes. During the process, new RNA polymerase sigma subunits appear, displacing one another and conferring on the RNA polymerase different specificities for the recognition of different classes of promoters. The activity of each sigma factor appears to be regulated in response to morphological cues that signal the completion of specific stages in the differentiation process, and in several instances intercellular signaling between the forespore and mother cell is required. Elucidation of the mechanisms that synchronize morphological transformations and gene expression in *Bacillus subtilis* may lead to the discovery of novel mechanisms that regulate gene expression in a wide variety of microorganisms. Therefore, we will study the mechanisms that synchronize the activation of two different sigma factors with two different morphological transformations. SapA protein acts in the early stage of spore coat assembly and may be proteolytically processed by the same machinery that processes pro- to activate thereby coordinating the activation of sigma K with the completion of the foundation for the spore coat. We will test this hypothesis, and determine how SapA is targeted to the interface region between the undercoat and cortex peptidoglycan. Forespore-specific transcription is initiated by sigma F-RNA polymerase, and results in the forespore-specific production of sigmaG. However, transcription of spoIIIG, the structural gene for , is delayed relative to other sigmaF-dependent genes, and requires an unidentified intercellular signal. We will characterize this mechanism of intercellular regulation by identifying cis-acting elements in the spoIIIG promoter, and trans-acting factors that regulate spoIIIG promoter activity. sigmaG accumulates after transcription of spoIIIG but does not become fully active until engulfment of the forespore is completed. We will study the function of two proteins that appear to affect sigmaG activation. Transcription of the orfD gene, which encodes one of these proteins, is directed from a promoter unlike any other known to be active during sporulation. Therefore, we will elucidate the mechanism that regulates orfD transcription. Mutations in spoIIIJ prevent sigmaG activation. We will test the hypothesis that spoIIIJ encodes a pheromone-like peptide that plays a role in signaling sigmaG activation.

Grant: 2R01GM055067-04
Program Director: JONES, WARREN
Principal Investigator: KENNELLY, PETER J
Title: REGULATORY DOMAINS OF PROTEIN PHOSPHATASE-1
Institution: VIRGINIA POLYTECHNIC INST AND ST UNIV BLACKSBURG, VA
Project Period: 1997/01/01-2004/03/31

Living cells must continuously sense and respond to a broad spectrum of intra- and extra-cellular inputs. This information must be processed so as to produce a response that is appropriate, comprehensive, and efficient. This complex task requires the intimate integration of signal transduction 'cascades' into computationally-sophisticated networks. Protein phosphorylation-dephosphorylation processes constitute prominent, core components of such networks. In mammalian cells the task of understanding regulatory networks as complete systems is confounded by the multiplicity (10³-10⁴) and redundancy of their components. A clear need exists for vehicles to permit study of the protein phosphorylation-dephosphorylation networks as integrated systems on a smaller scale in the near term. Such vehicles will serve as pathfinders for the analysis of more quantitatively complex organisms and will trace the history of their development. The objective of this proposal is to map the protein O-phosphorylation [i.e. those events targeting the hydroxyl side chains of serine, threonine, and/or tyrosine] network of the cyanobacterium *Synechocystis* sp. PCC 6803, a biochemically complex and environmentally adaptable organism. This cyanobacterium contains a quantitatively tractable protein O-phosphorylation network (approximately 10²) that prominently features homologs of 'eukaryotic' protein kinases, protein-serine/threonine phosphatases, and protein-tyrosine phosphatases. *Synechocystis* sp. PCC 6803 is genetically malleable and its complete genome sequence is known. The specific aims of the study outlined herein are: 1. To identify the proteins in *Synechocystis* sp. PCC 6803 that undergo modification via phosphorylation of serine, threonine, and/or tyrosine residues. 2. To identify the serine, threonine, and tyrosine-specific protein kinases and protein phosphatases in this organism. 3. To identify physiologically-relevant enzyme-substrate relationships between the phosphoproteins identified in aim 1 and the protein kinases and protein phosphatases identified in aim 2. The realization of these aims will contribute to our long-term goal of mapping and modeling the molecular interplay of a complete signal transduction/regulatory network on a cellular scale.

Grant: 1R01GM057215-01A1
Program Director: WEHRLE, JANNA P.
Principal Investigator: KOIDE, SHOHEI PHD
Title: STRUCTURE AND STABILITY OF SINGLE-LAYER BETA-SHEETS
Institution: UNIVERSITY OF ROCHESTER ROCHESTER, NY
Project Period: 2000/02/01-2004/01/31

The broad, long-term objective of the proposed project is to understand the basis of protein folding and stability. Beta sheets are a major structural component in proteins, but our understanding of the mechanism of beta-sheet formation is limited. Beta-sheet structures play critical roles in amyloid fibrils associated with serious diseases and in protein aggregation. The focus of the project is on the stability and folding of a novel, single-layer beta-sheet found in outer surface protein A (OspA) from *Borrelia burgdorferi*. OspA is a predominantly beta-sheet protein, and it contains, in the middle of the molecule, a three-stranded beta-region that is solvent-exposed on both faces. We showed that this single layer beta-sheet is stably formed in solution, and that the single-layer beta-sheet can be extended by inserting a beta-hairpin unit. Thus, our system based on OspA provides a unique opportunity to study the molecular mechanism of beta-sheet formation in the absence of extensive long-range interactions. Our specific aims are: 1) To characterize the equilibrium and kinetic folding mechanisms of OspA. 2) To identify factors contributing to the stability of the single-layer beta sheet. 3) To identify new amino acid sequences that stably fold into the single-layer beta-sheet conformation. 4) To characterize the structures of OspA fragments that contain the N-terminal globular domain and single-layer beta-sheets of various length. 5) To characterize the equilibrium and kinetic folding mechanisms of these N-terminal fragments. 6) To study amyloid-like fibril formation of synthetic peptides derived from the single-layer beta-sheet. To achieve these goals, we will apply biophysical techniques including NMR spectroscopy, small angle X-ray scattering and differential scanning calorimetry, protein engineering and combinatorial library screening. These studies will provide new insights into the stability and folding of beta-structure, and into the mechanisms of amyloid fibril formation. Outcomes of this study will also provide important information for structural genomics and de novo protein design.

Grant: 1R01GM058171-01A2
Program Director: WEHRLE, JANNA P.
Principal Investigator: SCHENCK, CRAIG C PHD
Title: MEMBRANE PROTEINS--STRUCTURE, DYNAMICS AND STABILITY
Institution: COLORADO STATE UNIVERSITY-FORT FORT COLLINS, CO
COLLINS
Project Period: 2000/02/01-2004/01/31

Membrane protein structures are vastly underrepresented in structure databases in relation to their importance and prevalence in living organisms. The investigators propose to develop and integrate novel liquids and solids NMR methods and biochemical methods (disulfide crosslinking and thermodynamic measurements) to determine the structure of a large membrane protein for the first time. LH1 protein, a 200 kD hetero-oligomeric membrane protein from photoheterotrophic eubacteria, undergoes multiple reversible unfolding (dissociation) and folding (association) reactions, and is well-suited for high-resolution structure determination by liquids and solids NMR. The proposed studies represent an important methodological proof-of-concept that will encourage structural investigations on other membrane proteins with direct medical relevance. The goal of the liquids NMR experiments is to determine the structures of LH1 subcomplexes and to ascertain the degree to which the individual solution structures can be used to build up structures of the intact complex. The solids NMR experiments will be used to determine structures of subcomplexes and intact complexes in a variety of solvent conditions, with the goal of determining NMR signatures for assessing membrane protein folding (association). Liquids and solids NMR dynamics will be ascertained for side- chain and backbone motions on a variety of timescales and they will be compared for LH1 and its subcomplexes. The dynamics will be related to the disulfide crosslinking kinetics, and to the thermodynamics of association. The overall goal is to understand the relationship between structure, conformational dynamics, function and thermodynamic stability for membrane proteins.

Grant: 1R01GM058560-01A1
Program Director: ANDERSON, JAMES J.
Principal Investigator: RUDD, KENNETH E AB
Title: TARGETED FUNCTIONAL GENOMICS AND PROTEOMICS OF E COLI
Institution: UNIVERSITY OF MIAMI-MEDICAL CORAL GABLES, FL
Project Period: 2000/02/01-2002/01/31

Abstract Text Not Available

Grant: 1R01GM058626-01A1
Program Director: WEHRLE, JANNA P.
Principal Investigator: ZOLKIEWSKI, MICHAL PHD
Title: STRUCTURE AND FUNCTION OF HSP 100 PROTEINS
Institution: KANSAS STATE UNIVERSITY MANHATTAN, KS
Project Period: 2000/05/01-2005/04/30

Failure of protein folding resulting in protein aggregation is a serious problem in molecular biology, biotechnology and medicine. Alzheimer's disease, prion diseases and inclusion-body myopathies are examples of pathological conditions arising from accumulation of misfolded and aggregated proteins in cells. Molecular chaperones, which belong to several heat-shock-protein (Hsp) families, inhibit protein aggregation and facilitate protein folding and assembly. Recently, novel multi-chaperone systems consisting of Hsp100, Hsp70, and Hsp40 proteins have been discovered in yeast and in *Escherichia coli*. Unlike previously studied chaperones, these multi-chaperone systems are capable of efficient reactivation of strongly aggregated proteins. In the *E. coli* system, ClpB, an Hsp100 protein with previously unknown function, cooperates with DnaK, DnaJ, and GrpE in reactivating aggregated luciferase. Our long-term goal is to understand the molecular mechanism of protein reactivation and disaggregation reactions involving Hsp100 proteins. In this proposal, we focus on ClpB as a member of the multi-chaperone system. ClpB is expressed in vivo as two gene products: 95-kDa ClpB95 and 80-kDa ClpB80. While both ClpB95 and ClpB80 exhibit ATPase activity, only the ATPase of ClpB95 is stimulated by other proteins. Our previous results show an ATP-induced self-association of ClpB, which is inhibited by ADP. We hypothesize that the mechanism of the ClpB chaperone function arises from allosteric couplings between ClpB-substrate of ClpB-co-chaperone interactions, ClpB self-association, and ATP binding and hydrolysis. We will test whether the observed differences in stimulation of the ATPase activities of ClpB95 and ClpB80 will be reflected in different protein-binding properties and chaperone activities of ClpB95 and ClpB80. The proposed research will achieve the following aims: 1. Characterize nucleotide-dependent oligomerization of ClpB95 ClpB80. 2. Characterize interactions between ClpB95 or ClpB80 and the co-chaperones (DnaK, DnaJ, GrpE). 3. Characterize the role of ClpB95 or ClpB80 and the three co-chaperones in the disaggregation and reactivation of luciferase.

Grant: 1R01GM058746-01A2
Program Director: CHIN, JEAN
Principal Investigator: KENNEY, LINDA J
Title: MOLECULAR CHARACTERIZATION OF PORIN GENE REGULATION
Institution: OREGON HEALTH & SCIENCE UNIVERSITY PORTLAND, OR
Project Period: 2000/04/01-2005/03/31

Two-Component regulatory systems have emerged as a paradigm for adaptive responses. The simplest systems consist of a sensor and a response regulator. The two-component system in *E. coli* that regulates the porin genes responds to changes in osmolarity of the growth medium. EnvZ, the osmosensor is phosphorylated by intracellular ATP and then phosphorylates OmpR. At low osmolarity, the major porin in the outer membrane is OmpF and at higher osmolarity, ompF is repressed and ompC is activated. A model arising from genetic studies predicts that phospho-OmpR (OmpR-P) binds with high affinity to activate ompF and with low affinity to repress ompF and activate ompC. Recent work by the PI indicates that this currently accepted hypothesis is not sufficient to account for porin gene regulation. This application contains three aims. The first is to use in vitro footprinting to examine the pattern of binding as a function of OmpR and OmpR-P concentration. If the binding is sufficiently different, it would lead to the rejection of the affinity hypothesis and alternatives would need to be considered. The aim further examines binding at low and high osmolarity both in vitro and in vivo, to correlate occupancy with osmoregulated expression of ompF and ompC. The second aim is to determine whether the linker of OmpR is required for communicating between the amino-terminal phosphorylation domain and the carboxyl-terminal DNA-binding domain and whether it plays an active or passive role in this process. If the linker is required, studies to determine the length and amino acid requirements (if any) will be conducted. The use of site-specific spectroscopic probes will explore the conformational changes that occur during signaling. The final aim is to determine the contact sites and to understand the interactions between OmpR and the alpha subunit of RNA polymerase (RpoA) that are important for transcriptional activation. This application begins with DNA-binding of OmpR and OmpR-P to the regulatory regions of ompF and ompC. It then focuses on the response regulator OmpR and examines conformational changes that are important for signaling. It lastly considers the role of OmpR interactions with RNA polymerase in stimulating transcription. Information gained in studying the porin regulon is relevant to systems in animal cells in which transmembrane signaling-dependent changes in kinase activity result in a phosphorylation cascade and ultimately to changes in gene expression. It is also relevant to pathogenic systems that use two-component systems to modulate their virulence properties.

Grant: 1R01GM059323-01A1
Program Director: CHIN, JEAN
Principal Investigator: HELMANN, JOHN D
Title: REGULATION OF MANGANESE BY MNTR IN BACILLUS SUBTILIS
Institution: CORNELL UNIVERSITY ITHACA ITHACA, NY
Project Period: 2000/02/01-2004/01/31

Abstract Text Not Available

Grant: 1R01GM059420-01A1
Program Director: SOMERS, SCOTT D.
Principal Investigator: GOYERT, SANNA M
Title: CHARACTERIZATION OF THE LPS RECEPTOR FOR ACUTE PHASE
Institution: NORTH SHORE UNIVERSITY HOSPITAL MANHASSET, NY
Project Period: 2000/06/01-2003/05/31

Sepsis due to Gram-negative infection remains a major cause of mortality. One of the earliest events occurring in a systemic infection is the acute phase response which has, as one of its major hallmarks, alteration of the concentration of plasma proteins (acute phase proteins, APP). APP are a set of functionally diverse proteins produced in the liver and defined in general as those proteins which show changes in plasma concentration (positive or negative) of 25 percent or more following the stimulus. APP are thought to increase host defenses as well as to control inflammation. There is a large body of evidence showing that cytokines (TNFalpha, IL-1 and IL-6) can induce the expression of APP. Since lipopolysaccharide (LPS, endotoxin), a component of the outer membrane of Gram-negative bacteria which is thought to be the major bacterial component of Gram-negative bacteria responsible for inducing the cascade of events leading to lethality in sepsis, stimulates both the production of TNFalpha, IL-1 and IL-6 as well as the production of APP, it has been reasonable to assume that the LPS induction of APP results from a secondary effect of cytokines secreted by macrophages when LPS stimulates them through the CD14-LPS receptor. To study the role of CD14 in the response to LPS, we have recently produced mice which lack the CD14-LPS receptor. These CD14-deficient mice produce little or no cytokines in response to very high concentrations of LPS; surprisingly however, they have a normal APP response. These observations indicate that mice have a non-CD14 receptor for LPS through which expression of APP is induced. Furthermore, as shown in Preliminary Studies, hepatocytes from CD14-deficient mice respond directly to LPS, indicating that this receptor is on hepatocytes. Accordingly, we propose to (1) study the binding characteristics of LPS and Lipid A to hepatocytes; determine whether their binding is specific and saturable; determine their binding constants (2) isolate and biochemically characterize the hepatocyte non-CD14 LPS receptor involved in the induction of the acute phase proteins using molecular methods of protein purification and gene cloning followed by functional verification and (2) determine the molecular mechanism(s) by which the LPS-APP receptor functions by comparing the genes induced via this receptor in hepatocytes to those induced by LPS via the CD14 receptor on monocytes/macrophages. These studies will not only clarify our understanding of the mechanisms involved in the induction of acute phase proteins by LPS, but will also increase our understanding of the pleiotropic effects of LPS and its various roles in sepsis.

Grant: 1R01GM059653-01A1
Program Director: MARINO, PAMELA
Principal Investigator: TIPTON, PETER A PHD
Title: ENZYMOLOGY OF ALGINATE BIOSYNTHESIS
Institution: UNIVERSITY OF MISSOURI COLUMBIA COLUMBIA, MO
Project Period: 2000/04/01-2004/03/31

DESCRIPTION: (Verbatim from the Applicant's Abstract): The proposed research program is a detailed investigation into the enzymology of alginate biosynthesis in the pathogenic bacterium *Pseudomonas aeruginosa*. *P. aeruginosa* infections are common and present significant health hazards to humans. Complications arising from colonization of lung tissues by *P. aeruginosa* are the leading cause of morbidity and mortality in cystic fibrosis patients. Alginate is a linear polysaccharide composed of mannuronate and guluronate residues, and is secreted by the bacteria to form an extracellular capsule, which contributes to their ability to effectively colonize lung tissue, resist antibiotic therapies and evade the host's immune system response. A potential strategy to combat *P. aeruginosa* infections is to develop agents which inhibit alginate biosynthesis and thereby render the bacteria susceptible to conventional antibiotics. Reports in the literature suggest that this strategy has merit, but to date, there are no effective specific inhibitors or inactivators of *P. aeruginosa* alginate biosynthetic enzymes. In order to approach the inhibition of alginate biosynthesis in a rational way, a deeper understanding of the functional properties and catalytic mechanisms of the constituent enzymes of the pathway is required. The research program described in the proposal focuses on C5 mannuronan epimerase and the enzymes which catalyze the first four steps of the alginate biosynthetic pathway. GDP-mannose dehydrogenase catalyzes the committed step in alginate biosynthesis, a mechanistically interesting four-electron oxidation, and will receive particularly close scrutiny. Detailed kinetic studies using transient kinetic approaches and kinetic isotope effect measurements will be performed in order to determine the energetics of the reactions; potential inhibitors and inactivators which have been designed based on hypotheses about the enzyme's chemical mechanisms will be characterized. The structure of phosphomannomutase, which catalyzes the second step in the pathway, will be determined by X-ray crystallography; GDP-mannose dehydrogenase has also been crystallized, and the determination of its structure will be pursued.

Grant: 1R01GM059690-01A1
Program Director: ANDERSON, JAMES J.
Principal Investigator: VISICK, KAREN L BS
Title: REQUIREMENTS FOR BACTERIAL COLONIZATION OF ANIMAL TISSUE
Institution: LOYOLA UNIVERSITY CHICAGO CHICAGO, IL
Project Period: 2000/04/01-2005/03/31

There is a fine line between symbiosis (mutualism) and pathogenesis. Most, if not all, animals are colonized by bacteria, many of which-the organism's "normal flora"-are tolerated or even encouraged. To establish a symbiotic relationship with an animal, a prokaryotic organism must be able to recognize and respond to a specific environment, establish communication with the host, colonize the host tissue, evade defense mechanisms, persist and multiply. Each of these steps is mirrored in pathogenic interactions, whether primary or opportunistic-and even normal flora have pathogenic potential in the event of excessive multiplication or introduction into areas they do not ordinarily inhabit. Despite the ubiquity of bacterium-animal symbioses, little is known about the genetic and molecular details of these interactions. In addition to increasing basic understanding of these important prokaryote-eukaryote alliances, the study of symbiotic interactions is expected to improve understanding of how pathogenic organisms adopt, subvert, or bypass the mechanisms leading to symbiosis and why they provoke dissimilar responses from the eukaryotic cell. The symbiosis between the bioluminescent marine bacterium *Vibrio fischeri* and its host, the squid *Euprymna scolopes*, provides an excellent opportunity to study the biology of a bacterium which can form a long- term, mutualistic association with an animal host. The symbiosis has been well-characterized at the organismal level. We now propose to begin the detailed molecular characterization of the interactions between these organisms, including establishment of the symbiotic state, signalling between the two organisms and developmental changes occurring in both partners as the symbiosis progresses. The proposed research will investigate the requirements for *V fischeri* colonization of host tissue, using a straightforward screen for transposon-insertion mutants defective in the initial symbiotic events. Identification and characterization of the critical colonization genes will lay the foundation for understanding signal exchange between a prokaryote and a eukaryote during the establishment of a long-term association.

Grant: 1R01GM059694-01A1
Program Director: SOMERS, SCOTT D.
Principal Investigator: WARREN, H SHAW
Title: BACTERIAL MEMBRANE PROTEINS IN GRAM-NEGATIVE SEPSIS
Institution: MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA
Project Period: 2000/03/01-2004/02/28

Gram-negative sepsis continues to cause substantial morbidity and mortality. Much of the pathophysiology is believed to be caused by toxic bacterial cell wall molecules, including lipopolysaccharides (LPS), that cause direct toxicity and/or induce a secondary pro-inflammatory response. Our preliminary data indicate that LPS is released from dying bacteria that blood in complexes that contain three conserved outer membrane proteins (OMPs), and that these OMPs may have biological activity themselves. The three bacterial membrane proteins have been identified as outer membrane protein A (OmpA), peptidoglycan associated lipoprotein (PAL), and murein lipoprotein (MLP). The overall objective of the proposal is to evaluate the role(s) of OmpA, PAL, and MLP in Gram-negative sepsis, with the ultimate goal of developing a strategy of treating sepsis with cross-reacting anti-OMP antibodies. Our first specific aim is to expand studies suggesting that there is a form of PAL released from the bacterial surface that floats at low density together with human lipoproteins. Our third specific aim is to study monoclonal and polyclonal IgG directed to epitopes on OmpA, PAL, and MLP for their ability to bind and neutralize the biological effects of each outer membrane protein and to promote opsonophagocytosis of LPS/OMP complexes. Our final specific aim is to study the protective efficacy of monoclonal and polyclonal immunoglobulin G directed to OmpA, PAL, and MLP in two models of Gram-negative infection. The studies should advance our fundamental understanding of the pathophysiology of Gram-negative sepsis. Each of the OMPs are highly conserved amongst clinical strains of Gram-negative bacteria. Therefore, a successful outcome of the project would provide a direct and immediate approach to developing anti-OMP antibodies for human use for the treatment of sepsis.

Grant: 1R01GM059721-01A1
Program Director: CHIN, JEAN
Principal Investigator: FOREST, KATRINA T BS
Title: TYPE IV PILI: STRUCTURAL ASPECTS OF THE ASSEMBLY
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 2000/06/01-2005/05/31

DESCRIPTION (Adapted from the Applicant's Abstract): Many pathogenic microorganisms have Type IV pili to attach to and colonize eukaryotic host cells for virulent infection. The type IV pili are long filamentous organelles comprised of thousands of copies of the pilin subunit. The long term objective of this work is to understand the molecular assembly mechanism of this virulence factor by solving the x-ray crystal structures of type IV pilins and the pilus biogenesis proteins. This information will ultimately be used to design inhibitors that block assembly, eukaryotic cell binding, and/or signaling by pili, thereby serving as antibiotics. In particular, during this funding cycle crystallization and x-ray structure determination will be done for the pilin subunit from *Pseudomonas aeruginosa* and the pilus assembly/motility factor PilT from the hyperthermophile *Aquifex aeolicus*. The subunit structure of pilin is needed to test the hypothesis that the 3-D subunit structure and oligomeric packing of type IV pili is conserved across species. It will also reveal posttranslational modifications. The structure of the pilin subunit will be used to model the subunit contacts in an assembled pilus fiber. Combined, the subunit structure and oligomer model will be the basis for design of hybrid pilin molecules to test hypotheses about which parts of the pilin molecule must interact specifically with each other and with other proteins in the biogenesis machinery. Two main roles of pili, twitching motility and signaling among bacteria and with eukaryotic cells, require the PilT protein. The PilT structure will reveal PilT surfaces likely to interact with other proteins in the biogenesis pathway. Logical site-directed mutants will be made to test the *in vivo* roles of these surfaces. Critical functional residues, effector binding sites in addition to the expected nucleotide binding pocket, and possible phosphorylation sites will furthermore be identified. Together with available biochemical and genetic data, these structural results will eventually lead to a high resolution model of the molecular mechanisms of pilus biogenesis and function. This understanding will be the foundation for blocking pilus functions to control infection by microbes.

Grant: 1R01GM060205-01
Program Director: SOMERS, SCOTT D.
Principal Investigator: WANG, STEWART C MD
Title: WOUND RESPONSE TO INFECTION: ROLE OF LPS-BINDING PROTEIN
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2000/09/01-2004/08/31

DESCRIPTION: Skin is the first line of defense against the hostile environment. Skin is not only a mechanical barrier but also an immune organ capable of mounting a coordinated inflammatory response. Inflammatory responses serve to recruit immune cells to the wound and also to contain and kill invading organisms. Lipopolysaccharide binding protein (LBP) is a potent facilitator of these functions and the investigators have found high levels of LBP bioactivity within surgical wounds. LBP at low levels potentiates cytokine production in response to LPS; these cytokines are critical in immune cell recruitment and activation. LBP is also an opsonin and potentiates the bactericidal activity of bactericidal/permeability increasing protein (BPI). Wound infections are commonplace after thermal injuries and represent a major cause of morbidity and mortality. Although systemic immune responses may contribute to local wound responses against infection, the investigators postulate that local factors within the wound, particularly LBP, play the most critical role. They hypothesize that LBP within the wound decreases bacterial infection after thermal injury by increasing local inflammatory cytokine production and bactericidal activity. The investigators propose to test this hypothesis by investigating the relationship between LBP, inflammatory cytokines and leukocyte bacterial killing in a rat model of cutaneous thermal injury. LPS is invariably present in burn wounds; LPS is also one of the most potent inducers of cytokine production by leukocytes and this action is markedly amplified by LBP. Aim 1 will determine the effect of wound LBP on local cytokine production after thermal injury. LBP functions as an opsonin for gram-negative bacteria and also reduces by 10,000-fold the concentration of neutrophil-derived BPI necessary to kill gram-negative bacteria. The investigators anticipate that increased LBP at the site of burn injury will increase wound bactericidal activity by potentiating neutrophil and macrophage recruitment, activation and bacterial killing. Aim 2 will determine the effect of wound LBP on local bactericidal activity after thermal injury. LBP present in the burn wound after injury could arise from a number of sources, of which transudation from the serum and local production are the two most likely. Wound derived LBP would be ideally situated to regulate the local immune response and may play an increasing role once altered capillary permeability after injury has resolved. Aim 3 will determine the source and regulation of wound LBP production after thermal injury.

Grant: 1R01GM060247-01
Program Director: RHOADES, MARCUS M.
Principal Investigator: STITT, BARBARA L PHD
Title: MECHANISM OF E. COLI TERMINATION FACTOR RHO
Institution: TEMPLE UNIVERSITY PHILADELPHIA, PA
Project Period: 2000/04/01-2004/03/31

Gene expression at the level of mRNA production is regulated both at the initiation and termination of transcription. Termination factor Rho is a homohexameric protein that releases newly synthesized RNA from Escherichia coli transcription complexes. Rho is thought to act through ATP-fueled, 5'->3' travel along nascent RNA. This travel is achieved by coordination of the RNA-dependent ATPase activity of Rho with RNA binding and release. Upon the arrival of Rho at the transcribing RNA polymerase, its continued travel unwinds the RNA-DNA helix of the transcription bubble, which disrupts the ternary transcription complex. The goal of the proposed work is the understanding the mechanism of directional travel by Rho along RNA. A fungal-developed anti-biotic, bicyclomycin, kills Gram-negative bacteria through Rho inhibition. A model in which Rho rolls along RNA as it hydrolyzes ATP predicts that ATP hydrolysis in the circularly arrayed Rho subunits will be sequential and in a fixed order. This prediction will be tested by using rapid mix/chemical quench techniques to examine pre-steady-state ATP hydrolysis kinetics. Other features of the kinetic mechanism will also be characterized, including determination of ligand binding order. Evidence for catalytic cooperativity among the active sites of Rho will be pursued and the requirements for cooperativity characterized. The rolling model also predicts that when RNA is bound to Rho, an unbound 5' region is the signal to Rho for ATP hydrolysis. This prediction will be tested by creating situations in which Rho has more or fewer than the usual number of signal RNA molecules bound. Each of these situations predicts changes in the pattern of ATP hydrolysis by Rho. This pattern will be monitored using rapid mix/chemical quench techniques and a combination of nucleotide binding with isotope partitioning experiments. Variant forms of Rho with characterized defects in RNA binding interactions will be used to clarify which of the two types of RNA binding sites on Rho interacts with the RNA signal for ATP hydrolysis. The proposed work will test and characterize the rolling model for ATP-dependent directional travel by Rho along RNA, which is the key to its transcription termination activity. The results will provide a better understanding of how the energy of ATP hydrolysis can be transduced to movement.

Grant: 1R01GM060268-01A1
Program Director: CHIN, JEAN
Principal Investigator: UHLENBECK, OLKE C PHD
BIOCHEMISTRY:PHYSICAL
Title: BIOPHYSICAL CHEMISTRY OF A DEAD/H PROTEIN
Institution: UNIVERSITY OF COLORADO AT BOULDER BOULDER, CO
Project Period: 2000/06/01-2004/05/31

DESCRIPTION: (From the Applicant's Abstract) The goal of this project is to obtain an understanding of the function of E. coli DbpA, a member of the large DEAD/H family of proteins that participate in many cellular pathways involving RNA. DEAD/H proteins couple the hydrolysis of ATP with RNA binding and are proposed to modify RNA secondary and tertiary structure. E. Coli DbpA and its B. subtilis homologue YxiN were chosen for detailed structural and mechanistic studies because, unlike nearly all other DEAD/H proteins, they bind tightly and specifically to a discrete region of 23S rRNA. The equilibrium and rate constants for the steps in the minimal kinetic scheme of the ATPase reaction will be determined to establish a framework for structure-function studies. The possibility that DbpA acts as a helicase in restructuring rRNA will be examined. RNA modification, photocrosslinking, and protein engineering experiments will test how different domains of DbpA interact with its cognate RNA, and whether the protein-RNA contacts change during the catalytic cycle. Finally, a DbpA disruption strain of E. coli will be used to search for the mechanism of action of DbpA in E. coli cells.

Grant: 1R01GM060610-01A1
Program Director: WEHRLE, JANNA P.
Principal Investigator: LEAR, JAMES D PHD
Title: BIOORGANIC MODELS FOR TRANSMEMBRANE PROTEINS
Institution: UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA
Project Period: 2000/09/30-2004/08/31

Description: (Applicant's abstract) The energetics governing the folding and function of membrane proteins is less well understood than for water-soluble ones. Thus, while there are abundant data concerning the free energy associated with the burial of an apolar group or the formation of a hydrogen bond in water-soluble proteins, such data are virtually non-existent for membrane proteins. We will determine how amino acid substitutions in the membrane protein bacteriorhodopsin affect its free energy of stabilization. It has been hypothesized that although water-soluble and membrane proteins differ greatly in the polarity of water-facing versus membrane lipid-facing residues, their interior side-chain packing and residue polarities are highly similar. To test this guiding hypothesis we will convert water-soluble proteins into membrane-soluble proteins and vice versa. Our specific aims, then, are: - Aim 1. Membrane-soluble versions of the water-soluble coiled-coil peptide, GCN4-P1, will be designed, synthesized and characterized in micelles and bilayers. - Aim 2. We will determine the effect of amino acid substitutions on the thermodynamic stability of bacteriorhodopsin. - Aim 3. We will design and characterize a water-soluble version of phospholamban, a 57-residue membrane protein that forms transmembrane pentamers. In each case, the thermodynamic properties of the proteins will be thoroughly examined to provide a deeper, quantitative understanding of membrane protein structure.

Grant: 1R01GM060632-01
Program Director: DEATHERAGE, JAMES F.
Principal Investigator: ROTHFIELD, LAWRENCE I MD
Title: E COLI MIN PROTEINS
Institution: UNIVERSITY OF CONNECTICUT SCH OF FARMINGTON, CT
MED/DNT
Project Period: 2000/02/01-2004/01/31

The long-range goal of this project is to determine the mechanism used by bacteria to select the proper division site at midcell in preference to other potential division sites that are located elsewhere within the cell. Since the products of the three min genes, minC, minD and minE, are responsible for this site-selection process, we will attempt to define the mechanism of action of each of the gene products in the site-selection process. Previous work has shown that MinD localizes to potential division sites at poles and midcell in the absence of the other Min proteins, and that coexpression of MinE or domains of MinE causes redistribution of membrane-associated Gfp-MinD into structures whose position depends on the presence of the MinE topological specificity domain. The major immediate aims of the proposal will be: i. To define the determinants in MinD that are responsible for its localization to poles and midcell, its role in formation of the MinE ring, and its ability to activate the MinC division inhibitor; ii. To determine the molecular basis of the different membrane-associated MinD structures that are induced by coexpression of Gfp-MinD with MinE and with the N-terminal region of MinE, using a combination of fluorescence microscopy, molecular genetics, and membrane-biochemistry; iii. To determine the localization pattern of MinC and the effects of MinC on localization of MinD and MinE; iv. To complete the high-resolution three-dimensional structure of the MinE topological specificity domain and to map topological specificity mutations of MinE to the three-dimensional structure; v. To characterize new minicell mutants that map outside of the minCDE locus and determine whether any of them code for the topological targets for MinD and MinE localization.

Grant: 1R01GM060662-01A1
Program Director: IKEDA, RICHARD A.
Principal Investigator: FLOSS, HEINZ G PHD CHEMISTRY:ORGANIC
Title: DEOXYHEXOSE BIOSYNTHESIS
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2000/06/01-2003/05/31

Many of the naturally occurring antibiotics and other biologically active microbial metabolites are glycosides, representing a combination of one of a large number of different aglycones with one or more of a number of unusual sugars, particularly deoxy- and deoxyaminohexoses. The sugar component in these compounds is usually absolutely essential for biological activity. Most of these sugar moieties are synthesized from D- glucose at the level of a dTDP-hexose and then transferred to the aglycone by a glycosyltransferase. Yet, except for the very initial steps, our knowledge of the mechanisms and enzymes involved in these sugar nucleotide transformations is limited. This limited knowledge has been an impediment to an attractive application in drug discovery, the combinatorial biosynthesis of unnatural assemblies of the same sugars and aglycones. In the proposed project we plan to enhance our understanding of the formation of key deoxyhexose components of antibiotics by reconstituting, with recombinant enzymes expressed from the appropriate cloned antibiotic biosynthesis genes, the enzymatic formation of sugar nucleotides representing dTDP-2-deoxy-, 2,3- dideoxy-, 2,3-dideoxy-3-amino-, and 3,4-dideoxy-3-aminohexoses and by studying the mechanisms of their formation. This work will lay one of the foundations for the combinatorial enzymatic synthesis of new, unnatural glycosides representing new combinations of antibiotic sugars and aglycones.

Grant: 1R01GM060715-01
Program Director: RHOADES, MARCUS M.
Principal Investigator: REDFIELD, ROSEMARY J PHD
Title: EVOLUTION OF DNA UPTAKE IN HAEMOPHILUS AND NEISSERIA
Institution: UNIVERSITY OF BRITISH COLUMBIA VANCOUVER, BC
Project Period: 2000/01/01-2003/12/31

DESCRIPTION (Adapted from the Applicant's Abstract): This project centers on DNA uptake sequences (USS) in *Haemophilus influenzae*. It includes a mixture of modeling experiments, genomic analyses and experimental work. Modeling will attempt to answer the question whether the benefits expected from the acquisition of nucleotides from other bacteria are sufficient to justify the evolution of bacterial competence for DNA transformation. In a separate approach, a model will be developed to test whether a receptor for DNA transformation with a sequence bias will result in the accumulation of USS sequences in the genome. Modeling will also examine the distribution of USS around the genome and attempt to determine whether it is random or not. Genomic analyses will be performed with genome sequences of *Actinobacillus actinomycetemcomitans*, *Neisseria gonorrhoeae* and *Neisseria meningitidis* to determine whether the same patterns of organization of USS apply to these organisms as to *H. influenzae*. These analyses will distinguish between single and repeated copies of USS and their distribution within and between ORFs. The genomes will also be scanned to determine whether they contain genes that are unusually homologous across species barriers. Experimental work will test whether killing by DNA transformation is restricted to *H. influenzae* strain Rd in order to determine whether lethality is a common phenomenon. Mucus contains DNA and therefore analyses will determine whether DNA can be taken up from mucus or not and whether the mucus that *H. influenzae* normally lives in inhibits transformation or not. The specificity of the USS will be tested by synthesizing 50mer USS that differ by one bp at each of 27 positions from the consensus sequence and testing them for uptake and ability to inhibit DNA uptake. Larger DNA fragments that differ in sequence will also be obtained by PCR amplification and tested. The absolute necessity of an USS for DNA uptake will be tested using longer incubation times than formerly used. Attempts will be made to identify a USS-binding protein on the bacterial cell surface and intracellularly. If a protein can be identified, it will be sequenced. Attempts will also be made to determine whether the USS are protected in nucleoids.

Grant: 1R01GM060731-01
Program Director: ECKSTRAND, IRENE A.
Principal Investigator: DYKHUIZEN, DANIEL E PHD
Title: AN EVOLUTIONARY ANALYSIS OF FIMBRIAE IN ENTEROBACTERIA
Institution: STATE UNIVERSITY NEW YORK STONY STONY BROOK, NY
BROOK
Project Period: 2000/02/01-2004/01/31

DESCRIPTION (Adapted from the Applicant's Abstract): The two principal investigators in this collaboration propose to study the evolution of a bacterium from a commensal to a pathogen through selection operating on pre-existing alleles, rather than through the acquisition of substantially different genes through horizontal transfer. (An example of the latter phenomenon is the "pathogenicity island" or a bacteriophage with a toxin gene.) The investigators use the term "pathoadaptation" for the process of selection of allele variants that "promote adaptation of the bacterial pathogens to a particular mode of pathogenesis." They propose to use for a model system type 1 fimbriae and adhesins of Escherichia coli and in particular the evolution of the adhesins for adaptation to the urinary tract and upper respiratory tract. For the study, the investigators will gather together representative strains and further characterize the fimbrial genes and the control regions for the genes. They will use Single Stranded Conformational Polymorphism and sequencing to distinguish the variants and to identify "signatures" for adaptation to the human host microenvironment in a survey type of study. In a prospective study they will use an animal model to test their hypothesis that variant alleles of fimbrial genes that promote persistence in the animal urinary tract will be selected and then detected in the population.

Grant: 1R01GM060759-01A1
Program Director: ECKSTRAND, IRENE A.
Principal Investigator: RICH, STEPHEN M PHD
Title: BORRELIA EVOLUTION AND POPULATION STRUCTURE
Institution: TUFTS UNIVERSITY BOSTON BOSTON, MA
Project Period: 2000/08/01-2005/07/31

The proposed project is designed to gain insight to the interactions and associations between *Borrelia* pathogens and their respective zoonotic vertebrate reservoirs and arthropod vectors. To fully characterize the structure of natural populations of *B. burgdorferi*, it is necessary to sample isolates from the various levels of its organization to determine the temporal, geographic, and host environmental factors which determine the distribution and exchangeability among *B. burgdorferi* isolates. These geographic requirements of this sampling are met by the use of coastal islands, which have been chosen for the unique qualities that make them ideal natural laboratories for tracking the ecological dynamics of emerging disease. The temporal sampling of these two sites is accomplished both longitudinally (by sampling each island bi-annually for each year of the study), and by cross-sectional sampling of the tick cohorts in non-overlapping annual cycle. The relationship between the host environments can be tested directly, since the *Borrelia* isolates are collected directly from their hosts. The multi-locus genotypes of all mice, ticks, and *Borrelia* isolates from each island site (and at each time point) will be determined, thus providing a rich resource for evaluating the structure of their respective populations, and the interactions among these species.

Grant: 1R01GM060791-01A1
Program Director: MARINO, PAMELA
Principal Investigator: MORRIS, J G
Title: POPULATION BIOLOGY OF SURFACE POLYSACCHARIDES IN CHOLERA
Institution: UNIVERSITY OF MARYLAND BALT PROF SCHOOL BALTIMORE, MD
Project Period: 2000/07/01-2004/06/30

Cholera is an ancient disease in the midst of a modern resurgence, with cases reported to WHO by 65 member nations in Asia, Africa, the Americas, and Europe in 1997. With the appearance of *V. cholerae* 0139 Bengal and the recent identification of epidemic-associated 037 strains, there is increasing recognition that modifications in bacterial surface polysaccharides play a key role in the emergence of new, epidemic-associated strains. While the number of constituent sugars appears to be relatively small (less than 25), there are hundreds of O antigen/capsule types recognized among *V. cholerae* and other Vibrionaceae strains. Initial phylogenetic studies suggest that serotype and/or capsule type are independent of phylogeny. We hypothesize that there is frequent horizontal/sexual transfer and rearrangement of surface polysaccharide biosynthetic gene cassettes in *V. cholerae*, providing an opportunity for periodic emergence of new, pandemic cholera strains. Making use of our collection of Vibrionaceae (including the Smith Vibrio Reference Laboratory/serotype collection) and ongoing strain acquisition, we propose: 1) To describe and define the genetic relatedness of a 192-strain *V. cholerae* test collection, utilizing sequence analysis of conserved genetic elements (multilocus sequence typing) including genes used in multilocus enzyme electrophoresis/zymovar analysis. 2) To identify and characterize *V. cholerae* O antigen and capsular polysaccharides for strains within this collection, as a basis for assessing horizontal transfer of surface polysaccharide genes. This will include a) determination of sugar composition of O-antigen side chain and capsule; and b) identification and correlation of sugars with specific biosynthetic genes/gene combinations. 3) To assess the extent of genetic variability in surface polysaccharides plausibly available to *V. cholerae*, and, in particular, to *V. cholerae* strains with pandemic potential. For these latter studies we will a) correlate genetic relatedness (as determined above) with transfer/acceptance of specific polysaccharide genes; b) determine the placement and organization of biosynthesis genes for the sugars of the O side chain and capsule; and c) assess the nucleotide sequence divergence of the most commonly identified polysaccharide biosynthesis genes. Taken together, these studies will substantively expand our understanding of the evolutionary biology of *V. cholerae*, giving us a much better appreciation of the potential for changes in *V. cholerae* surface polysaccharides and, in turn, for the emergence of *V. cholerae* strains which can form the basis for new cholera pandemics.

Grant: 1R01GM060793-01
Program Director: ANDERSON, JAMES J.
Principal Investigator: BESSEN, DEBRA E PHD
Title: EVOLUTION OF GROUP A STREPTOCOCCI
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 2000/02/01-2004/01/31

DESCRIPTION (Adapted from the Applicant's Abstract): The population genetic structure of group A Streptococcus pyogenes will be investigated by multi locus sequence typing (MLST) of a globally representative sample of 600 strains using 7 distinct gene loci. Data from an additional 200 strains will have been obtained with other funding prior to the beginning of this project. 276 bacteria will be selected for MLST to represent the diversity of emm sequence typing (emmst), of which 146 types are currently known, and from as diverse sources and time periods as possible. Additional emmst will be performed. The data will be analyzed by cluster analysis of differences in alleles, indices of association, and phylogenetic trees to determine how important the role of recombination is. The speA and speC alleles of the individual strains will also be tested. The population distributions of the M-protein and the housekeeping genes tested by MLST will be compared to determine how well they correlate. These analyses will test the hypothesis that the M protein has led to more discrete strains in the emm pattern A-C subpopulation than in the emm pattern D strains. A second hypothesis is that in emm pattern E strains, two sets of polymorphic antigens (emm plus sof) elicit immune responses and immunity has selected strains that do not overlap in these antigens (Gupta's two epitope model). To test this hypothesis, polyclonal antisera will be generated against overlapping recombinant sof polypeptides and the type-specific sites for binding mapped by determining which overlapping region reacts in the different recombinant peptides. That type-specific region will be sequenced from all pattern E isolates. Two-way contingency tables will be used to determine whether the combinations of emmst and sof alleles are as predicted by the hypothesis or random. Emm-based binding sites for human IgG subclasses, IgA, plasminogen and fibrinogen will be screened by Southern hybridization and the data will be analyzed similarly to above. An additional 325 strains will then be chosen based on the results from the above analyses to analyze short term epidemiology from defined situations. The same analyses will be performed as with the first set of strains. Finally, the antisera to emm and sof products will be analyzed by competitive inhibition, opsonization and for inhibition of binding of ligands to the bacteria. Cross 0- reactivity between different M proteins will be correlated with the phylogenetic analyses and models for selection by immunity. Additional antisera will be obtained and tested after immunization with type-specific epitopes of emmst and sof gene products. These sera will be tested to determine whether strain structure is determined by the immunogenicity of the emm and sof gene products.

Grant: 1R01GM060795-01
Program Director: ECKSTRAND, IRENE A.
Principal Investigator: KEIM, PAUL S PHD
Title: ANTHRAX MOLECULAR EVOLUTION, DIVERSITY AND EPIDEMIOLOGY
Institution: NORTHERN ARIZONA UNIVERSITY FLAGSTAFF, AZ
Project Period: 2000/05/01-2004/04/30

DESCRIPTION (Adapted from the Applicant's Abstract): The overall goal of this study is to link the molecular genetic evolution of *Bacillus anthracis* to epidemiological models of North American and African anthrax in an effort to better understand the geographic, biotic, and abiotic conditions responsible for outbreaks of anthrax in the world at large and the sources of this pathogen. Towards this end, Dr. Keim and his collaborators will: (1) study the genetic-molecular epidemiology of *Bacillus anthracis* isolated from different geographic locations and habitats, (2) ascertain the different factors (parameters) that affect the distribution of the molecular markers they employ in their epidemiology surveys and estimate the values of these parameters in laboratory culture. (3) Using Monte Carlo simulations, they will ascertain how the distribution of these markers within and between populations of *Bacillus anthracis* will be affected by geographic, genetic and ecological processes.

Grant: 1R01GM060997-01
Program Director: LEWIS, CATHERINE D.
Principal Investigator: XU, ZHAOHUI PHD
Title: MOLECULAR RECOGNITION IN BACTERIAL PROTEIN TRANSLOCATION
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2000/03/01-2005/02/28

Protein translocation is one of the fundamental aspects of cell biology. Understanding how proteins move from their sites of synthesis to their sites of action is relevant since almost half of the proteins of a cell are transported into or across a membrane. In fact, the amounts and locations of particular proteins are controlled during development, during the cell cycle, and for maintenance of healthy cells, failure in secretion results in not only activity deficiency at the indeed subcellular location but also toxic levels of molecules in the wrong place. The long term objectives of the following proposal are to understand the molecular mechanisms by which cellular machinery translocates proteins across membrane. The current focus is on the early events that occurs in the E. coli Sec translocation system, with emphasis on mechanisms regarding (1) the binding of a polypeptide by a translocation dedicated chaperone SecB (2) the general recognition motif within the translocating polypeptide, and (3) the interaction of SecB with its membrane receptor SecA and the effect of polypeptide binding by SecB. Our approach will be to use high resolution X-ray crystallography to establish the three- dimensional structures of SecB and its relevant complexes with peptides and/or SecA. Mutational and biochemical experiments will then be used to complement structural studies. The combination of these approaches will help us to understand the physical chemistry that govern protein translocation by the Sec system.

Grant: 1R01GM061019-01
Program Director: MARINO, PAMELA
Principal Investigator: YOUNG, KEVIN D
Title: COMPLEX PHENOTYPES OF MULTIPLE MUTANTS OF E COLI
Institution: UNIVERSITY OF NORTH DAKOTA GRAND FORKS, ND
Project Period: 2000/03/01-2004/04/30

Some biological traits are mediated by combinations of genes or proteins whose interactions are so complicated that we can not predict an organism's phenotype even with an intimate knowledge of its genotype. The twelve penicillin binding proteins (PBPs) of *Escherichia coli* form a model system for the study of such "complex phenotypes." The PBPs synthesize, modify and maintain the rigid peptidoglycan layer of the bacterial cell wall and are the targets of our most important single class of antibiotics, the beta-lactams. Nonetheless, despite decades of work, we do not know the detailed biological functions of these enzymes nor can we describe the biochemical pathways by which they operate. This information is becoming increasingly important with the rise of antibiotic resistant organisms. Our long term objective is to explain the structure, synthesis, and function of bacterial peptidoglycan so that more rational antimicrobial strategies can be devised. Therefore, we constructed 192 *E. coli* strains from which were deleted every possible combination of eight different PBPs. This comprehensive set of mutants allowed us to show that such a combinatorial genetic strategy produces results impossible to classic genetic approaches. Preliminary screening of the mutants revealed unusual and unanticipated phenotypes, including: capsule production, morphological aberrations, phage resistance, resistance to antibiotic-induced lysis, and temperature sensitivity. In most cases, the traits did not appear in cells with fewer than three or four mutations, and these phenotypes depended in a complex way on the combinations of active PBPs. We propose to complete the screening of this set of mutants for traits likely to be affected by alterations in the peptidoglycan-e.g., in antibiotic-or chemically-induced autolysis, phage resistance, protein secretion, and in the morphogenesis of extracellular structures. In addition, analytical techniques will be adapted so that phenotypic predictions can be made from knowledge of the genotype in complex situations. Three significant results can be anticipated. First, we will identify new phenotypes in basic cellular processes in which the PBPs and peptidoglycan play fundamental biological roles. Second, we will understand better how the PBPs maintain the bacterial cell wall and how beta-lactam antibiotics induce its destruction. And third, the compilation of extensive and defined datasets will allow us to develop appropriate tools to investigate complex relations between genotype and phenotype.

Grant: 1R01GM061028-01
Program Director: SHAPIRO, BERT I.
Principal Investigator: BLOUNT, PAUL L PHD PHYSIOLOGY, OTHER
Title: MOLECULAR MECHANISMS OF MECHANOSENSITIVE CHANNEL GATING
Institution: UNIVERSITY OF TEXAS SW MED DALLAS, TX
CTR/DALLAS
Project Period: 2000/06/01-2005/05/31

Bacterial mechanosensitive channels are emerging as molecular paradigms for investigation of the mechanosensory transduction that occurs in physiological processes such as touch, proprioception, cardiovascular regulation, hearing and balance. The use of the MscL channel of *Escherichia coli* has advanced the field considerably by allowing molecular genetic analysis to be combined with electrophysiology and transport assays in the study of a protein with a well-defined physiological role. Hence, MscL serves as a good model for determining the molecular mechanisms of mechanosensitive channel gating as well as the general principles of how proteins detect and respond to membrane tension. Purification of the MscL channel protein led to the identification of the structural gene and ultimately to a 3.5 angstrom X-ray crystallographic structure of the *Mycobacterium tuberculosis* MscL. This is a closed structure, which unfortunately provides few clues to the process of channel gating or to the structure of the open channel. Because a large pore of 30 to 40 angstrom diameter is generated upon gating, a large conformational change must occur and several residues normally embedded in protein or lipid environments must contribute to the lining of the open pore. The residue interactions that are strongest and of greatest importance for keeping the channel closed, the movements of residues and domains that take place upon gating, and the residues that contribute to the lining of the open pore are currently all unknown. The experiments in this application are designed to use molecular analyses based on the solved structure to determine these functional and structural properties of the channel. The approaches include: 1) Cysteine scanning to determine which interactions within the transmembrane domains are of most importance in MS channel gating. 2) Utilizing the "Substituted Cysteine Accessibility Method" (SCAM) to identify the residues that move into an aqueous environment upon channel opening, and thus are likely candidates for lining the open-channel pore. 3) Isolating and characterizing suppressor mutations of gain-of-function MscL mutants to identify pairs of residues that potentially interact in the closed, transition or open states.

Grant: 1R01GM061074-01
Program Director: DEATHERAGE, JAMES F.
Principal Investigator: MARGOLIN, WILLIAM BS
Title: TARGETING AND ASSEMBLY OF E COLI CELL DIVISION PROTEINS
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX
HOUSTON
Project Period: 2000/09/01-2005/08/31

Cell division is essential for proliferation of bacteria, and appears to rely on a molecular machine positioned at the site of division. Despite extensive knowledge of the major proteins involved in this fundamental cellular process, very little is understood about how these proteins are targeted, how they assemble into a complex, and how they radically alter normal cell wall growth in order to synthesize the division septum. The long term goals of this project are to elucidate the molecular mechanisms behind the targeting and assembly of cell division proteins, using *Escherichia coli* as a model system and taking advantage of new, powerful cytological methods for bacteria. A key universal cell division protein, FtsZ, self-assembles into a polymeric ring (the Z-ring) at the surface of the inner membrane at the division site and recruits other proteins, including FtsA, ZipA, and a group of integral membrane proteins, all of which help to complete the septum. The first aim of this proposal is to gain a molecular understanding of the recruitment of FtsA and ZipA by FtsZ. One way that this will be addressed is by mutagenesis of a region of FtsZ that is involved in FtsZ-FtsA and FtsZ-ZipA interactions in order to obtain proteins defective in interaction. Once such mutants are isolated, selection for intragenic and extragenic suppressors may reveal residue-specific contacts. Novel fluorescent in situ protein interaction assays will be used to screen for protein-protein interactions, followed by biochemical tests for direct interaction. The second aim will investigate the role of FtsA in cell division. Enzymatic and structural properties of purified FtsA will be characterized. In addition, the ability of FtsA to interact with FtsZ, ZipA, and downstream cell division proteins in the membrane will be tested. The final aims of the project are to understand how the Z-ring gets targeted to its exact cellular address. The nucleoid appears to negatively influence Z-ring assembly, and it is possible that nucleoid segregation releases a negative signal to allow precise Z-ring positioning. Studies of Z-ring assembly in mutants defective in nucleoid structure and chromosome replication will be carried out to determine the relationship between the nucleoid and the Z-ring. Finally, the Min system, which consists of MinC, D, and E, is an important regulator of Z-ring assembly and positioning. The relationship between the Min system and FtsZ will be addressed in detail both biochemically and genetically. The results of this proposed research are expected to provide a more complete understanding of the molecular mechanisms of cell division, and should facilitate the isolation of new and better antimicrobial drug targets.

Grant: 1R01GM061099-01
Program Director: JONES, WARREN
Principal Investigator: ALLEN, KAREN N BS BIOLOGY
Title: STRUCTURAL AND MECHANISTIC STUDIES OF PHOSPHONATASE
Institution: BOSTON UNIVERSITY MEDICAL CAMPUS BOSTON, MA
Project Period: 2000/04/01-2005/03/31

DESCRIPTION (applicant's abstract): The studies proposed in this new grant application will examine the structure and mechanism of action of the enzyme phosphonatease and extend structural and mechanistic studies to other members of the haloacid dehalogenase (HAD) enzyme superfamily. Each enzyme of this superfamily uses a conserved Asp residue to form either an acylphosphate-enzyme intermediate or an alkyl ester-enzyme intermediate. This chemistry is supported by a common structural scaffold. Phosphonatease catalyzes the hydrolysis of phosphonoacetaldehyde (P-Ald) to acetaldehyde and orthophosphate. In conjunction with 2-aminoethylphosphonate transaminase, phosphonatease functions in a two-step biodegradative pathway used to recycle P, N, and C from the ubiquitous natural phosphonate, 2-aminoethylphosphonate. Despite the wide range of known biological activities associated with natural and synthetic phosphonates, the enzymology of phosphonate metabolism is poorly characterized. The goal of these studies is to derive an understanding of the process of enzyme catalyzed C-P bond cleavage using phosphonatease as the model system. The first set of experiments proposed will test mechanistic models based on the recently determined phosphonatease X-ray structure (Allen laboratory) and on previous mechanistic studies (Dunaway-Mariano laboratory). Site-directed mutagenesis coupled with transient kinetic analysis will be used to test the chemical steps of the models. Crystallographic structure determinations carried out on dead-end complexes formed using substrate analogues, enzyme mutants, and chemically modified enzymes will be used to capture the structures of proposed reaction intermediates. The second set of experiments proposed will examine the active-site diversification of the HAD enzyme superfamily. The ability of phosphonatease to catalyze the reactions of other family members will be determined and protein engineering will be used to swap catalytic activities of two family members. To further probe the catalytic plasticity of the superfamily, the structure and mechanism of beta-phosphoglucomutase, a phosphotransferase from the HAD family will be examined. The goal of these studies is to derive an understanding of how the enzyme superfamily active site has been adapted to catalyze C-X, P-O and C-P bond cleavage in a variety of different substrate structures.

Grant: 1R01GM061145-01
Program Director: DEATHERAGE, JAMES F.
Principal Investigator: BLAIR, DAVID F PHD
Title: STRUCTURE-FUNCTION STUDIES OF FLAGELLAR ROTOR COMPONENTS
Institution: UNIVERSITY OF UTAH SALT LAKE CITY, UT
Project Period: 2000/04/01-2004/03/31

Many of bacteria swim by rotating helical filaments that act as propellers. This motility is a factor in the virulence of many bacterial pathogens, including those that cause ulcers, syphilis, burn wound infections, and some diarrhea. Each filament is driven by rotary motor in the cell membrane; the filament/motor structure is called a flagellum. The energy for rotation comes from the membrane iron gradient. Like any rotary motor, the bacterial flagellar motor possesses a stator (the non-rotating part) and a rotor (the rotating part). FliG, FliN, and FliM are three proteins that function in a complex on the rotor. Recently, x-ray crystallography has been used to determine the three-dimensional structure of a domain of the rotor protein FliG. This domain functions directly in motor rotation, and is known to interact with proteins of the stator. This is the first high-resolution structure determined for any component of the flagellum. In the work proposed here, the FliG domain structure will be exploited to guide detailed biochemical and functional studies of this key rotor component. Another flagellar rotor protein, FliN, has also been crystallized and preliminary data show that it will be feasible to determine its structure. The structure of FliN will be determined, and also used to guide biochemical and functional studies. The long-term goal of this work is to understand the structure of the protein complex that forms the flagellar rotor, and to understand the mechanism of motor rotation in light of this structure. The proposed work will bring us significantly nearer this goal, by revealing structures and spatial relationship of rotor components in unprecedented detail.

Grant: 1R01GM061162-01
Program Director: SOMERS, SCOTT D.
Principal Investigator: LEWIS, KIM A PHD
Title: A GENOMICS APPROACH TO P. AERUGINOSA BIOFILMS
Institution: TUFTS UNIVERSITY MEDFORD MEDFORD, MA
Project Period: 2000/09/01-2001/06/30

Description (Adapted from the applicant's abstract): The PI's long-term objectives are to understand the mechanism of microbial biofilm resistance to antibiotics, and to develop effective anti-biofilm therapies. This is a collaborative project that brings together the expertise of the PI in microbial multidrug resistance and the expertise and advanced genomics tools to study *P. aeruginosa* that have been developed by the co-Investigator and colleagues at PathoGenesis. The mechanism of extremely high resistance of microbial biofilms to antibiotics is poorly understood. Data from the literature and the PI's own findings indicate that biofilm cells might be expressing antibiotic resistance mechanisms. Finding genes responsible for biofilm resistance to antibiotics is the main goal of this application. There are two specific aims. In Specific aim 1, the PI will identify genes specifically expressed in biofilms. Two groups of experiments are proposed. (a) In vitro studies. Biofilm RNA will be isolated, labeled and used to probe a *P. aeruginosa* DNA array. A detailed analysis of factors likely to affect expression of biofilm genes will be performed. These factors will include the age of the biofilm, various growth conditions, including those emulating growth in vivo, and growth in the presence of antibiotics which might induce resistance. (b) In vivo studies. Sputum samples that have been collected from patients with cystic fibrosis harboring *P. aeruginosa* biofilm infections will be used to isolate RNA and obtain gene expression profiles. This information will allow the PI to identify biofilm-specific genes that are expressed in vivo. In Specific aim 2, the PI proposes to validate candidate genes expressed in the biofilm. Three types of experiments are proposed. (a) Mutants from an ordered Tn insertion library will be used to test involvement in antibiotic resistance of genes whose expression is changed in biofilms. Each candidate mutant will be tested in detail with a representative panel of antibiotics. This experiment will show which specific biofilm genes are responsible for increased resistance. In a complementary approach, a complete Tn insertion library will be screened for mutants with increased antibiotic susceptibility. Candidate mutants from this screen will then be tested in detail with a larger panel of antibiotics at a broad range of concentrations. (b) Possible "Universal" resistance genes will be identified by comparison with genes expressed in *E. coli* biofilms and probed with an *E. coli* DNA array. (c) *P. aeruginosa* mutants in genes participating in antibiotic resistance will be used to obtain expression profiles. This information will likely contribute to the understanding of the mechanism of resistance. Resistance genes identified in this project will serve as targets for drug discovery and development of effective anti-biofilm therapies.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01GM061308-01
Program Director: SOMERS, SCOTT D.
Principal Investigator: ORR, SALLY L PHD
Title: EXPRESSION CLONING COMPONENTS OF THE LPS RECEPTORS
Institution: SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA
Project Period: 2000/05/01-2002/04/30

Systemic Inflammatory Response Syndrome (SIRS) or septic shock from bacterial infection, trauma or burn injury can be fatal. Over one-half of septic shock cases are a result of gram-negative bacterial infection. Structure/function studies of gram-negative lipopolysaccharide (LPS) have revealed a group of compounds that act as LPS antagonists. Lipid Iva is an LPS precursor molecule that has a unique species-specific bioactivity. Lipid Iva is an LPS antagonist in human cells and exhibits full LPS agonist activity in murine cells. This species-specific difference in bioactivity is believed to be a result of the differences in structure between the murine and human LPS signaling receptor. We used the species-specific difference to isolate, by expression cloning, a molecule that may be involved in LPS signaling. Our preliminary results describe the isolation and DNA sequence analysis of a partial murine cDNA (G21) that enables human cells to respond to lipid Iva. In addition we demonstrate that a stable human cell line containing the murine cDNA responds to lipid Iva stimulation in a CD14-dependent manner. Therefore this murine cDNA may be a part of the LPS recognition complex. Our goal for the two years of this proposal is to isolate and characterize both the murine and human full-length cDNAs encoding this receptor. These cDNAs will be used to construct stable cell lines to confirm lipid Iva and LPS dependent induction of inflammatory mediators (TNFalpha, ICAM-1). In vitro mutagenesis studies will be employed to identify the areas(s) involved in ligand recognition. If this receptor is part of the innate immune response to bacteria, we will have a new target for the design of drugs to fight septic shock.

Grant: 1R01GM061336-01
Program Director: ANDERSON, JAMES J.
Principal Investigator: REILLY, JAMES P PHD
Title: STALK SYNTHESIS PROTEINS OF CAULOBACTER
Institution: INDIANA UNIVERSITY BLOOMINGTON BLOOMINGTON, IN
Project Period: 2000/05/01-2004/04/30

Abstract Text Not Available

Grant: 1R01GM061753-01
Program Director: MARQUEZ, ERNEST D.
Principal Investigator: TREPANIER, LAUREN A DVM
Title: ROLE OF HYDROXYLAMINE REDUCTION IN DRUG HYPERSENSITIVITY
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 2000/07/01-2005/06/30

Sulfamethoxazole (SMX), sulfadiazine, dapsone, pentamidine, and procainamide are clinically important drugs, the use of which is limited by the occurrence of hypersensitivity reactions in some patients. These reactions are thought to be related to the presence of a toxic hydroxylamine metabolite and its spontaneous byproduct, the nitroso metabolite. Unlike the parent compounds, these metabolites are both cytotoxic and immunogenic. Hypersensitivity reactions to SMX and related drugs occur in a high percentage (25-60 percent) of patients with AIDS. The reason for this high incidence is unclear, but data from in vitro cytotoxicity assays in peripheral blood mononuclear cells (PBMC's) suggest that both HIV-positive and HIV-negative patients with such hypersensitivity have a defect in hydroxylamine or nitroso detoxification. We hypothesize that detoxification of hydroxylamine and nitroso metabolites by reduction is an important determinant of hypersensitivity to sulfonamide and related drugs. We further hypothesize that NADH cytochrome b5 reductase, which is involved in hydroxylamine reduction in some species, is important for hydroxylamine detoxification in humans. In addition, we have novel data to suggest that flavins (FAD and FMN); in the presence of glutathione, are capable of non- enzymatic reduction of hydroxylamines. We therefore propose that defects in either NADH- or flavin-dependent reduction of hydroxylamines and nitroso metabolites are associated with the outcome of sulfonamide hypersensitivity. To address these hypotheses, we will examine the role of NADH cytochrome b5 reductase (b5R) in hydroxylamine and nitroso reduction in both liver and PBMC's, using expressed human recombinant b5R and antibodies to human b5R. We will evaluate the role of flavins in hydroxylamine and nitroso reduction using in vitro stoichiometric assays in a cell-free system, and correlation of flavin and glutathione content with activity in hepatic microsomes and PBMC's. Finally, we will correlate hydroxylamine and nitroso reduction, b5R expression, and flavin content with the outcome of SMX hypersensitivity in patients with HIV infection. The ultimate goal of these studies is to better understand the pathogenesis of toxicity to sulfonamides and a related group of clinically important drugs which generate hydroxylamine and nitroso metabolites. The results of these experiments, which will identify the pathways involved in the detoxification of these metabolites, will suggest better strategies for the prevention of hypersensitivity and other hydroxylamine-related toxicity.

Grant: 1R01GM061761-01
Program Director: IKEDA, RICHARD A.
Principal Investigator: BOONS, GEERT-JAN PHD
Title: SYNTHESIS AND EVALUATION OF NOVEL ENDOTOXIN ANTAGONISTS
Institution: UNIVERSITY OF GEORGIA ATHENS, GA
Project Period: 2000/07/01-2005/06/30

This project is a strategic component of our long-range research goals, which are to identify the molecular mechanisms responsible for the contribution of endotoxin (LPS) to septicemia, and to use this new knowledge to develop efficacious treatments. The project has four objectives designed to test our central hypothesis that structurally unique lipid-A analogs based on the lipid-A of nitrogen-fixing rhizobial bacteria exert antagonistic effects on enteric LPS-induced production of proinflammatory mediators by interfering with the interaction between enteric LPS and CD14 receptors on human monocytes. To test this hypothesis, an initial series of twenty-seven uniquely hybrid lipid-A analogs having structural characteristics ranging from the classic lipid-A of *E. coli* to that of the rhizobial species will be obtained by organic synthesis. These well defined, pure analogs then will be tested for agonist activity on human monocytic cells, using the production of tumor necrosis factor (TNF) activity as the primary readout. These structure/function analyses will permit identification of individual structural alterations in lipid-A that result in the presence or lack of agonist activity. The analogs then will be tested for their ability to antagonize the pro-inflammatory effects of enteric LPS in classical pharmacologic antagonism studies and for their ability to prevent binding of radiolabeled enteric LPS to the monocytes. The latter studies will be performed to evaluate the involvement of CD14- dependent and CD14-independent pathways. Finally, studies will be performed to determine the relative roles of the NF B and MAP kinase pathways in transducing the effects of the lipid-A analogs. Completion of these studies will provide the basis required to identify the structural features responsible for the deleterious effects of lipid-A and the beneficial effects of potentially novel LPS antagonists. On completion of this project, we will have important information about a potentially novel approach to prevent the proinflammatory effects of enteric LPS. The rationale for these studies is that until the molecular mechanisms responsible for LPS activation of mononuclear phagocytes are understood and the most efficacious antagonists are identified, it will not be possible to prevent LPS-induced complications of septicemia.

Grant: 1R01GM061887-01
Program Director: EDMONDS, CHARLES G.
Principal Investigator: LAIBLE, PHILIP D. BS
Title: A BACTERIAL FACTORY FOR PRODUCTION OF MEMBRANE PROTEINS
Institution: UNIVERSITY OF CHICAGO CHICAGO, IL
Project Period: 2000/08/01-2004/07/31

Description: (verbatim from the applicant's abstract) Membranes and the proteins embedded within them compartmentalize specialized machinery that provides the means by which cells and organelles communicate, generate energy, take up nutrients, excrete wastes, transduce signals by transporting metabolites between internal compartments, and build gradients of ions (and other small molecules) which are used to fuel all normal cellular activities in healthy organisms. Structural information for membrane proteins is exceedingly scarce - it is notoriously difficult to purify quantities of native material that are sufficient for crystallization attempts. Today's methods for the overproduction of protein cannot deal with membrane proteins, thus this important class is virtually ignored. In order for significant advances to be made, innovative strategies are needed rather than incremental advances in existing technology. We have exploited the unique physiology of the Rhodobacter species of photosynthetic bacteria to overexpress heterologous proteins, and have recently shown that a human outer membrane protein is expressed and incorporated into induced membranes of this organism. We now propose to develop this system to be a general one for the expression of functional membrane proteins - from any organism - in quantities that are sufficient for biochemical studies and crystallization trials for structure determination.

Grant: 1R01GM061980-01
Program Director: SCHWAB, JOHN M.
Principal Investigator: KELLY, T R PHD
Title: THE SYNTHESIS OF LACTONAMYCIN AND ANALOGS
Institution: BOSTON COLLEGE NEWTON, MA
Project Period: 2000/06/01-2004/05/31

The objectives of this project are to develop an efficient synthesis of the recently discovered molecule lactonamycin and to prepare analogs of it that optimize its biological activity. Lactonamycin's hexacyclic structure is unlike that of any other compound. It exhibits potent activity against bacteria resistant to existing antibiotics including methicillin-resistant (MSRA) and vancomycin-resistant (VRE) strains. Lactonamycin also possesses significant anticancer activity. In the course of our work we intend to explore new general strategies for achieving stereo-, regio-, and enantioselective transformations. The specific aims are: To develop a short and efficient synthesis of lactonamycin. To use that effort as an opportunity to explore new general strategies for accomplishing stereo-, regio-, and enantiocontrolled synthetic transformations including (i) extending the role of intramolecular hydrogen bonding and/or borate complexation in Diels-Alder reactions, (ii) developing a dynamic thermodynamic (as opposed to kinetic) resolution, and (iii) examining new ligands for controlling intramolecular delivery of OsO₄ under circumstances where Sharpless-type asymmetric dihydroxylation is expected to fail. To employ the synthetic route to prepare analogs of lactonamycin with the aim of (i) making available water-soluble derivatives of lactonamycin, (ii) separating and optimizing the antibiotic and antitumor activities, (iii) identifying the pharmacophores, and (iv) beginning SAR studies. To test, in collaboration with Pfizer Inc., analogs and synthetic intermediates for antibiotic, anticancer and other biological activities.

Grant: 1R01GM061988-01
Program Director: LONG, ROCHELLE M.
Principal Investigator: YAN, BINGFANG DVM
Title: SIGNALING OF PREGNANE X RECEPTOR
Institution: UNIVERSITY OF RHODE ISLAND KINGSTON,, RI
Project Period: 2000/07/01-2004/06/30

DESCRIPTION (Adapted from the applicant's abstract): Cytochrome P4503A (CYP3A) enzymes involve the metabolism of two thirds of drugs and other xenobiotics. Induction of CYP3A enzymes by many compounds is known as an important contributing factor to many failures of therapy or severe toxicity. CYP3A induction is featured by marked species difference, structural diversity of the inducers, and inter-individual variation. Analyses of CYP3A promoters locate three cis-response elements likely involved in CYP3A induction. A reporter gene construct containing one of the elements can be transactivated by an orphan receptor designated the pregnane X receptor (PXR), and the differential activation of mouse and human PXR by several compounds largely reflects the species difference observed in vivo. The central hypothesis of the proposed studies is that PXR plays a determinant role in CYP3A induction and multiplicity/polymorphism, along with inducibility of PXR, are responsible for the species difference, inducer diversity and individual variation. The specific aims of this project are: (1) to determine the multiplicity/polymorphism of PXR in humans; (2) to determine the essentiality of PXR in the CYP3A induction; (3) to determine the synergistic effects of PXR inducers on PXR activator-mediated CYP3A induction; and (4) to determine important residues of PXR in conferring CYP3A induction by rifampicin (RIF) and pregnenolone 16 α -carbonitrile (PCN). As part of the studies to determine the molecular basis for the existence of multiple forms and polymorphic variants of PXR in humans, a cDNA-trapping method will be used to screen cDNA libraries from hepatic and extrahepatic tissues. Transient cotransfection experiments with a CYP3A reporter will be conducted to determine the activation profile of each PXR. To determine the essentiality of PXR in CYP3A induction, PXR antisense constructs will be tested for their ability to block CYP3A induction; and PXR chimeras with a ligand binding domain from another species will be tested for their ability to modulate CYP3A expression in response to species-selective activators. To determine the synergistic effects of PXR inducers on PXR activator-mediated CYP3A induction, rats and hepatocytes will be treated with PXR inducers, PXR activators, or in combination; induction of CYP3A will be determined by Northern and Western blot analyses. Site-directed mutagenesis experiments will be conducted to determine functionally important residues of PXR in conferring CYP3A induction by RIF and PCN. Significant progress has been made toward the proposed objective. Full-length cDNAs encoding multiple forms of rodent and human PXR have been isolated. Several compounds are found to drastically increase rPXR-1 mRNA levels. These results support our hypothesis that multiplicity and polymorphism along with inducibility of PXR are responsible for species difference, inducer diversity and individual variation featured by CYP3A induction.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01GM062117-01
Program Director: FLICKER, PAULA F.
Principal Investigator: KERN, DOROTHEE PHD
Title: NMR STUDIES OF BACTERIAL SIGNAL TRANSDUCTION
Institution: BRANDEIS UNIVERSITY WALTHAM, MA
Project Period: 2000/05/01-2005/04/30

Bacterial signal transduction is predominated by two-component systems. These systems consist of two proteins, an autophosphorylating histidine kinase and a response regulator, which is activated by phosphorylation at an aspartate residue in a Mg^{2+} dependent reaction. Because of their crucial role for the survival of bacteria and lower eukaryotes and its high homology, two component systems are attractive as potential new targets for antimicrobials. In addition, these systems control the expression of virulence and drug resistance factors in several pathogenic organisms. Inhibition of the two component pathway may present an opportunity to depress resistance by targeting multiple proteins with a single inhibitor. When phosphorylated, the receiver domain ("switch" component of the response regulator) modulates the activity of its cognate output domain, often a transcriptional activation domain. No structure has been obtained for the phosphorylated form of either an isolated receiver domain or an intact response regulator due to the short half-life of the phospho-aspartate linkage. A long-term goal of this laboratory is to elucidate the mechanism of activation of response regulators using the transcriptional activator NtrC (nitrogen regulatory protein C) as model system. NtrC consists of three domains, the N-terminal receiver domain, the transcriptional activation domain and the DNA-binding domain. First, the structure of the transiently phosphorylated receiver domain will be determined by NMR. The main tricks used are (a) creating a steady state using large excess of phosphodonor and (b) adding multiple three dimensional data sets taken on multiple NMR samples. Second, the mechanism of activation triggered by phosphorylation will be characterized by NMR relaxation experiments and amino acid substitutions that uncouple phosphorylation and activation. The active site structure will be probed by heterologous metal ion replacement. Third, the signal cascade from the receiver domain to the transcriptional activation domain will be investigated. This problem is challenging because of the size of the full-length protein (104 kDa). Methods for segmental isotopic labeling using the splicing enzymes inteins will be combined with recently developed NMR techniques such as TROSY and dipolar couplings in liquid crystalline medium.

Grant: 1R01GM062750-01
Program Director: TOMPKINS, LAURIE
Principal Investigator: GOLLNICK, PAUL D PHD BIOCHEMISTRY
Title: REGULATION OF THE TRYPTOPHAN GENES IN BACILLUS
Institution: STATE UNIVERSITY OF NEW YORK AT AMHERST, NY
BUFFALO
Project Period: 2000/09/15-2004/08/31

Controlling transcription termination prior to the coding region is a commonly used strategy to regulate gene expression in bacteria, including many with importance to human health. Such control mechanisms are collectively termed attenuation and antitermination. The proposed research will investigate the mechanisms by which RNA binding proteins recognize and bind to specific sites in RNA, and how these interactions regulate transcription attenuation. The model system of study is the TRAP protein (trp RNA-binding Attenuation Protein), an RNA binding protein that regulates transcription attenuation of the tryptophan biosynthetic genes in *Bacillus subtilis* and related Bacilli. In the presence of excess tryptophan, TRAP is activated to bind to a series of 11 GAG or UAG repeats in the 5' leader region of the trp operon. This binding induces formation of a transcription terminator, which halts expression of the genes. TRAP is an 11 subunit protein that forms a symmetric ring. RNA binds to TRAP by wrapping around the outer perimeter of the protein ring. The detailed mechanism by which TRAP associates with its RNA target will be characterized using a combination of equilibrium binding studies, nucleoside analogs, and rapid-quench stopped-flow studies. TRAP is activated to bind RNA by binding 11 molecules of L-tryptophan. Crystallography, genetics and biochemical approaches will be used to determine the mechanism by which tryptophan binding activates TRAP. The third objective is to develop a more detailed understanding of the mechanism of TRAP mediated transcription attenuation. Key elements of the TRAP binding site in the trp leader will be altered and the effects of these changes on attenuation studied in vivo using a trpE"-lacZ gene fusion. These studies will be guided by the information learned from in vitro studies of the TRAP/RNA interaction.

Grant: 1R01GM063244-01
Program Director: SOMERS, SCOTT D.
Principal Investigator: FINBERG, ROBERT W
Title: CD14 Signaling and Activation of Innate Immune Response
Institution: UNIV OF MASSACHUSETTS MED SCH WORCESTER, MA
WORCESTER
Project Period: 2000/09/01-2004/08/31

DESCRIPTION (Adapted from the Applicant's Abstract): The proposed studies are focused on detailed characterization of the molecular mechanism of CD14-dependent activation of immunocompetent cells during sepsis and septic shock. CD14 is a membrane GPI-anchored "pattern recognition receptor" that is found to be associated with sphingolipid-rich microdomains in monocytes and macrophages. Although CD14 lack of a transmembrane domain, it is capable of recognizing a variety of gram-negative and gram-positive bacterial components including but, perhaps, not limited, lipopolysaccharide (LPS), peptidoglycan, lipoarabinomannan (LAM), and lipoproteins. This CD14-mediated activation of monocytes/macrophages by bacterial products results in a potent host cytokine response. Evidently, specific binding of microbial constituents to CD14 initiates a signal transduction cascade in the target cells. The potential role of CD14 in the pathophysiologic sequelae of bacterial sepsis is supported by studies on CD14 knockout mice that strongly indicate the resistance of these mice to otherwise lethal LPS-mediated shock and to the effects of severe bacterial infection. However, the mechanism of CD14-dependent activation of phagocytes remains poor understood. A number of Toll-like receptors (TLRs), that are homologues to the *Drosophila* Toll receptor, have recently been characterized. It was shown that TLRs mediate signaling by LPS and other microbial constituents in mammalian cells. The PI and his colleagues have demonstrated a direct association between Toll-like receptors and LPS in the cell membrane. To define the functional role of CD14 and TLRs in signaling and activation of myeloid cells, it is proposed to use a combination of biochemical (delineation of CD14-TLR associated kinase cascade) and genetic approaches (selection of mutant macrophage cells and correction of their specific defects with a cDNA library). The relationship between CD14, scr kinases, G proteins and the TLRs in mechanism of signaling will be defined, as will cellular location. These studies will help to define how host invasion by microbes leads to septic shock and may provide novel targets for the design of therapeutic interventions against the life-threatening conditions associated with bacterial sepsis.

Grant: 1R01GM063270-01
Program Director: ANDERSON, JAMES J.
Principal Investigator: BLASER, MARTIN J MD INTERNAL
MED:INFECTIOUS DISEASE
Title: MATHEMATICAL MODELS OF H PYLORI GASTRIC COLONIZATION
Institution: NEW YORK UNIVERSITY SCHOOL OF NEW YORK, NY
MEDICINE
Project Period: 2000/09/01-2004/08/31

DESCRIPTION (Adapted from the Applicant's Abstract): *Helicobacter pylori*, a gram negative bacteria that colonize the human stomach, are associated with diseases of the upper gastrointestinal tract. Examination of the selective pressures on *H.pylori* provide a model for host interactions with resident flora since (I) *H.pylori* strains are highly diverse, and continued variation is occurring during colonization of a single host; (ii) *H.pylori* strains lack SOS repair, are naturally competent, and have substantial ability to exchange DNA with other *H.pylori* cells, providing opportunities for the observed "quasi-species" development; (iii) *H.pylori* Lewis expression varies during colonization and host characteristics selected for particular phenotypes; (iv) molecular loci have been identified that are involved in Lewis variation, and mouse models have been developed in which variation can be predictably observed; (v) Mathematical models have been developed for *H.pylori* colonization in general and the dynamics of Lewis expression can be measured and develop a model to predict its behavior. The specific aims are (1) To construct an experimental rodent model for assessing population genetic changes in *H.pylori* during gastric colonization of a single host, and to develop a deterministic mathematical model that is representative and predictive. (2) To assess the effects on variation of prior host phenotype, inoculum size, bacterial mutation rate, and host immunological response on *H.pylori* population dynamics, phenotypes, and genotypes to better identify the parameters of the mathematical model. (3) To examine the effect of recombination between two different *H.pylori* strains during experimental infections, and to develop representative mathematical models for this more complex phenomenon.

Grant: 2R15GM054280-02
Program Director: CHIN, JEAN
Principal Investigator: GARRETT, JANETTA M MOTH
Title: YEAST AMINO ACID PERMEASES
Institution: HAMILTON COLLEGE CLINTON, NY
Project Period: 1996/06/01-2004/05/31

Yeast amino acid permeases are responsible for transporting their substrates into the cell in response to the external environment and the nutritional requirements of the cell. A family of yeast amino acid/auxin permeases has been identified through the Yeast Genome Project and the substrate range and regulation of many of these permeases has been elucidated. Through the study of these permeases, increasing evidence has accumulated that there are groups of permeases with distinct, and limited, metabolic roles in the cell. For example, it appears that these permeases can be classified as anabolic or catabolic in function and that anabolic specific permeases are not capable of supplying their amino acid substrate for growth. The mechanism of differentiation of function of these highly homologous permeases is not understood at present. Two possible mechanisms are presented by which an anabolic transporter could provide an amino acid for protein synthesis but not for growth: signaling or channeling. The experiments outlined in this proposal are designed to elucidate whether an amino acid permease can form part of the signaling pathway that indicates the availability of amino acid as well as transporting it into the cell. We will also investigate whether different types of permease contribute their substrate to the free amino acid pools of the cell or whether certain transporters 'channel' their substrate into a particular metabolic fate. These studies will be initiated focusing on tryptophan and its anabolic permease, Tat2. Tryptophan uptake is of particular interest because there are many unusual phenotypes associated with low tryptophan levels in yeast. There are also interesting parallels between yeast tryptophan uptake and auxin response in plants.

Grant: 1R15GM060261-01
Program Director: SCHWAB, JOHN M.
Principal Investigator: LI, GUIGEN PHD
Title: ASYMMETRIC REACTION PROCESSES
Institution: TEXAS TECH UNIVERSITY LUBBOCK, TX
Project Period: 2000/05/01-2003/04/30

DESCRIPTION: (Applicant's Abstract) Development of new synthetic approaches to alpha-(aminoalkyl)acrylates and alpha-(hydroxyalkyl)acrylates and related analogs has become increasingly important for the synthesis of numerous chemically and biologically important molecules. The application of alpha-(aminoalkyl)acrylates to the design and synthesis of anti cancer drug taxol/taxotere has already provided interesting and promising results. Beta-nonsubstituted (aminoalkyl)acrylates can be synthesized by using the Baylis-Hillman reaction in which there are serious limitations such as slow reaction rate and narrow scope. Since beta-substituted acrylate derivatives do not undergo the Baylis-Hillman reaction, beta-substituted (aminoalkyl)acrylate and alpha-(hydroxyalkyl)acrylate analogs cannot be obtained by use of the Baylis-Hillman process. This proposal is intended to develop novel asymmetric additions of alpha-functionalized vinyl anions to carbonyl derivatives for the synthesis of both beta-nonsubstituted and beta-substituted (aminoalkyl)acrylates and (hydroxyalkyl)acrylates. The new asymmetric processes will be directed by chiral auxiliaries, chiral reagents and catalysts. The push and pull strategies incorporating Bronsted Lewis acids and bases will be applied in the catalyst selections. Reaction conditions such as concentrations of reactants and catalysts, cofactors, solvents or cosolvents, and reaction temperature will be systematically investigated for the proposed project. The new processes will be applied to the design and synthesis of anti cancer drug taxol/taxotere analogs.

Grant: 1R15GM061307-01
Program Director: ANDERSON, JAMES J.
Principal Investigator: JAYASWAL, RADHESHYAM K
Title: REGULATIONS OF IRON ACQUISITION BY S AUREUS
Institution: ILLINOIS STATE UNIVERSITY NORMAL, IL
Project Period: 2000/04/01-2002/06/30

DESCRIPTION (Adapted from the Applicant's Abstract): *Staphylococcus aureus* is a major cause of human diseases, ranging from basic skin abscesses to complicated life-threatening diseases such as toxic shock syndrome, pneumonia, septicemia, and infective endocarditis. The acquisition of broad antibiotic resistance in this bacterium, especially the methicillin and vancomycin-resistant *S. aureus* strains, poses a major threat to public health. The virulence factors involved in the pathogenicity of *S. aureus* have been extensively studied, but the molecular mechanisms governing *S. aureus* invasion, colonization, and survival within the host environment remains largely unknown. Various stress conditions within the host are proposed to simulate the expression of virulence genes in *S. aureus*. Iron, which has been shown to be an important virulence determinant and required for many vital cellular processes, is not readily available for bacterial cells in the animal host. To cope with an iron-deficient host environment, the bacteria have evolved several mechanisms of iron acquisition and its regulation. However, these pathways are poorly understood in *S. aureus*. The PI has recently cloned and partially characterized a *S. aureus* chromosomal gene, *fur*, that shows homology to the ferric-uptake regulatory genes of other bacteria. The *fur* gene codes for a protein that binds to a specific DNA sequence in the promoter region of at least two genes, *sirA* and *fhu*. The long-term goal of this project is to investigate how *Fur* regulates iron acquisition in *S. aureus* and its role in the regulation of the expression of virulence genes. The specific objectives of this study are 1) cloning and molecular analysis of *fur*, 2) construction of a mutation in *fur* and determination of its effect on pathogenicity, 3) molecular analysis of *Fur*, and 4) characterization of the *Fur* regulated iron uptake operon, *fhu* of *S. aureus*. To accomplish this first objective, the PI has cloned *fur* which will be overexpressed and the protein purified to conduct gel-shift and footprint assays. Northern blots will be done to determine the mechanism of *fur* regulation. To accomplish the second objective, *Fur*- mutants will be generated by site-directed mutagenesis and the effect of the mutation on pathogenicity will be determined using a rat model of endocarditis. The *Fur*-mutant and the parent will be used to identify *Fur*-regulated genes in an in vitro cycle selection procedure or alternatively by 2-D gel electrophoresis to accomplish the third objective. The fourth objective will be accomplished by sequencing the *fhu* operon, followed by the construction of a mutation in each reading frame to determine their roles in the pathogenicity and uptake of iron. The results of this study may identify novel targets that interfere with iron uptake and its regulation and may lead to alternative therapies for staphylococcal infections.

Grant: 1R15GM061310-01
Program Director: JONES, WARREN
Principal Investigator: SUMMERS, RICHARD G PHD
Title: ENZYMATIC DETERMINANTS OF ERYTHROMYCIN STEREOCHEMISTRY
Institution: LAWRENCE UNIVERSITY APPLETON, WI
Project Period: 2000/05/01-2001/08/31

DESCRIPTION (adapted from applicant's abstract): The proposed research focuses on the biosynthesis of the clinically important antibiotic erythromycin. It is the long-term goal of this project to produce novel erythromycin derivatives that cannot be prepared by traditional chemical syntheses. New antibiotic derivatives such as these are urgently needed, particularly in light of the increased threat posed by newly emergent antibiotic resistant bacteria. In specific, this work seeks to determine the enzymatic domains that dictate the stereochemistry of the erythromycin macrolactone ring and then use this knowledge to genetically engineer the antibiotic producing bacteria, *Saccharopolyspora erythraea*, to produce new erythromycin derivatives. Currently, the genes for the erythromycin synthase have been cloned, and much is known about the biosynthesis of this chemically complex antibiotic. Yet, the enzymatic domains responsible for the stereochemical configuration of ten distinct sites in the erythromycin macrolactone ring are unknown. Since it has already been shown that the erythromycin synthase can be altered to produce new erythromycin derivatives through genetic engineering, knowledge of the determinants of erythromycin stereochemistry should enable the production of entirely new series of antibiotic derivatives, many of which may be biologically active. Indeed, just through alterations in stereochemistry, over a hundred new erythromycins are theoretically accessible. The approach to be taken here centers initially on the in vitro construction of genetic chimeras encoding altered erythromycin synthases using standard recombinant DNA techniques. These altered synthases will feature enzymatic domain interchanges focusing on those domains most likely involved in the determination of erythromycin stereochemistry (i.e. a domain thought to produce one stereochemical outcome will be replaced with an analogous domain thought to produce the opposite stereochemical outcome). Once the genetic chimeras have been constructed in vitro, the wild type genes of the natural erythromycin-producing organism will be replaced (via a two step gene replacement protocol) and the erythromycin derivatives produced by the mutant organisms will be isolated and characterized by NMR. Importantly, most of the work proposed here will be conducted by undergraduate chemistry and biology majors, consequently this research project will also provide an ideal training opportunity for students interested in medical biotechnology and genetic engineering.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R15GM061318-01
Program Director: DEATHERAGE, JAMES F.
Principal Investigator: ROBINSON, JAYNE B PHD
Title: QUORUM SENSING IN BACTERIAL TWITCHING MOTILITY
Institution: UNIVERSITY OF DAYTON DAYTON, OH
Project Period: 2000/04/01-2003/12/31

Pseudomonas aeruginosa is a ubiquitous and important opportunistic pathogen that possesses an impressive arsenal of virulence factors. Two quorum-sensing systems, *las* and *rhl*, have been identified as controlling production of several of these virulence factors and has become a model for studying cell-density control of virulence genes. Evidence that the quorum-sensing systems of *P. aeruginosa* play a major role in twitching motility is presented here for the first time. Recently, *P. aeruginosa* has also become the preferred model system for studies of type-4 pili and twitching motility. The polar, type-4 pili of *P. aeruginosa* are multifunctional structures which play a crucial role in the virulence of this organism by promoting adherence to, and colonization of, a variety of tissues as well as being the organelles responsible for twitching motility. The overall goal of this research is to work out the cellular and molecular basis for the role of quorum sensing in the flagella-independent, pili-associated mode of bacterial surface translocation known as 'twitching motility' in *P. aeruginosa*. A primary goal of this project is to identify the twitching motility gene(s) that are controlled by the quorum-sensing systems of *P. aeruginosa*. The cellular and molecular mechanisms of twitching motility are not well understood, nor is it clear how the quorum-sensing systems affect these mechanisms. The mechanisms underlying twitching motility, and its connection to quorum-sensing will be addressed at the genetic, biochemical and cell-cell interaction levels. Finally, the contributions quorum-sensing and twitching motility make to the frequency of conjugal mating in *P. aeruginosa* on semi-solid surfaces will be examined for the first time. Standard plate matings between wild-type cells and the various twitching motility and quorum-sensing mutants will be conducted. In addition, the temporal and spatial nature of gene transfer among members of the populations on semi-solid surfaces will be studied at the single cell level using synthesis of GFP to follow conjugal transfer.

Grant: 1R15GM061690-01
Program Director: ANDERSON, JAMES J.
Principal Investigator: GOODNER, BRADLEY W PHD
Title: GENOMICS APPROACH TO A PATHOGEN OF ANIMALS AND PLANTS
Institution: UNIVERSITY OF RICHMOND RICHMOND, VA
Project Period: 2000/07/01-2001/06/30

Agrobacterium tumefaciens is a well-known plant pathogen, but has only recently been recognized as an opportunistic pathogen of humans. More information is needed to understand how the bacterium can be such a broad range pathogen, both for understanding its basic biology and to provide possible targets for future control of human infection. The specific goals of this proposal are 1) to functionally characterize two recently discovered genes that have strong homology to known virulence or interaction determinants in other organisms, 2) to determine if *A. tumefaciens* really contains a remnant of an animal-specific virus in its genome as a "footprint" of a past animal infection event, 3) sequence about 10 percent of the *A. tumefaciens* genome in order to search new genes involved in virulence or interaction, and 4) to functionally characterize a few of these new genes. Both biochemical and genetic approaches will be used to test gene functions.

Grant: 2R37GM029498-20
Program Director: CHIN, JEAN
Principal Investigator: LANYI, JANOS K
Title: LIGHT-DRIVEN ION TRANSPORT IN BACTERIAL RHODOPSINS
Institution: UNIVERSITY OF CALIFORNIA IRVINE IRVINE, CA
Project Period: 1981/07/01-2005/06/30

Bacteriorhodopsin and halorhodopsin are the simplest ion pumps, and prototypes of the ubiquitous seven-transmembrane-helical proteins. We have made unusual progress in the past years and now propose to test and extend the resulting detailed step-by-step hypothesis for the mechanism of proton transport. It includes not only the proton transfer steps in the protein but also the thermodynamic and structural rationales for the unidirectionality and the vectoriality of the ion translocation. All aspects of this comprehensive hypothesis are now open to critical examination, and in the proposed work we will pay particular attention to the numerous mutant phenotypes not predicted by its present version. We will continue to use our present approach of combining sitespecific mutagenesis, time-resolved spectroscopy, and high-resolution protein x-ray crystallography to investigate i) structural questions at the proton release step, ii) how the pKs of dissociable groups are modulated, iii) the nature and causes of conformational coupling, iv) the mechanism of proton transport in the absence of asp-85 and asp-96, and v) the lessons to be learned from halorhodopsin and the D85T bacteriorhodopsin mutant, that both transport chloride ions and in the opposite direction from protons. The goal of this proposal is to establish, finally, a full and explicit model for the molecular mechanism of this kind of ion pump.

Grant: 2R37GM029764-20
Program Director: DEATHERAGE, JAMES F.
Principal Investigator: LUTKENHAUS, JOSEPH F PHD
Title: REGULATION OF CELL DIVISION
Institution: UNIVERSITY OF KANSAS MEDICAL CENTER KANSAS CITY, KS
Project Period: 1981/07/01-2005/06/30

The long-term objectives of this research are to determine the molecular mechanisms involved in bacterial cell division and the underlying spatial and temporal regulatory mechanisms. Our efforts have focused on the FtsZ protein which assembles into a cytoskeletal ring that recruits other proteins to the division site and directs cell division in bacteria. Recent work has shown that FtsZ is a structural and functional homologue of the eukaryotic cytoskeletal protein tubulin. Like tubulin FtsZ undergoes dynamic assembly that is regulated by GTP hydrolysis. Also, FtsZ is a target of several inhibitors that regulate cell division in bacteria. Recent work has shown that SulA and MinC are inhibitors of FtsZ assembly. SulA is induced in response to DNA damage and MinC is part of the division site selection system. In the present proposal biochemical and genetic studies are designed to determine the mechanism of action of these inhibitors. At present SulA is thought to sequester FtsZ monomers and MinC is thought to destabilize FtsZ polymers. Our present studies should further define the interaction between FtsZ and these inhibitors to test the postulated mechanisms. Also, several aspects of MinC's mode of action will be investigated including its interaction with MinD and MinE, which cause it to oscillate between the poles of the cell. In addition, studying the various mutant FtsZs should reveal additional aspects of FtsZ assembly. The present proposal will also examine the interaction between FtsZ and ZipA and FtsA. Genetic and biochemical experiments are proposed to investigate the interacting surfaces between these proteins and the role of these proteins in FtsZ assembly. FtsZ mutants that will be isolated will serve as controls for in vitro experiments. Additional ftsZ(Ts) mutations will be isolated and used to look for suppressors in an attempt to find other proteins that regulate FtsZ assembly. Research over the past few years has shown that FtsZ is a universal feature of bacterial cell division. It has great similarity to tubulin but is also quite distinct. As a result it should prove to be a useful, novel target for antimicrobial therapy.

Grant: 2P01HD032652-06
Program Director: OSTER-GRANITE, MARY LOU
Principal Investigator: WHITLEY, CHESTER B PHD
Title: GENE THERAPY FOR METABOLIC DISORDERS
Institution: UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN
Project Period: 1995/01/10-2002/12/31

The central theme of this program is to exploit recent advances in basic science for the development of innovative gene therapy strategies, especially for metabolic disorders causing mental retardation. The program is highly focused on identifying and resolving the barriers to clinical gene therapy. In this period, five projects aim to exploit recent innovations that would enhance gene delivery and expression or actually correct genomic mutations in vivo. These projects are: Therapy for Hyperammonemia with Genetically-Engineered Bacteria (Tuchman). Adeno-Associated Virus Vector Treatment of Spinocerebellar Ataxia (McIvor) Chimeraplasty for Mutations Associated with Mental Retardation (Kren) Sleeping Beauty Transposon for Gene Therapy (Hackett) Lentiviral Ex Vivo Hematopoietic Stem Cell Gene Therapy for Mucopolysaccharidosis Type 1 (Whitley) The projects utilize a number of models of human metabolic disorders causing brain disease, notably, murine models of mucopolysaccharidosis, hyperammonemia, phenylketonuria and spinocerebellar ataxia. The program share core facilities for administration, microchemicals, quantitative PCR, hematopoietic cell processing, animal resources and viral vector production.

Grant: 1R01HD038559-01A1
Program Director: WRIGHT, LINDA
Principal Investigator: JORDAN, JEANNE A PHD
Title: DETECTING NEONATAL SEPSIS USING MOLECULAR TECHNIQUES
Institution: MAGEE-WOMEN'S HOSPITAL OF UPMC PITTSBURGH, PA
Project Period: 2000/08/16-2004/05/31

DESCRIPTION: (Adapted from the Investigator's Abstract): Approximately 150,000 to 500,000 infants greater than 34 weeks gestational age are admitted to NICUs annually who are at risk for systemic infection. These infants all receive systemic antibiotic therapy. However, the vast majority of these infants are not infected, but rather have symptoms that are secondary to other medical conditions. The current standard of care for evaluating neonatal sepsis is blood culturing, which lacks sensitivity and is not informative prior to at least 24 to 46 hours. As a result, there is prolonged use of antibiotic therapy in many newborns that could be shortened if a more rapid test to rule out sepsis was available. This application proposes to investigate two potential tests, a PCR-based amplification assay for detecting bacterial 16S ribosomal DNA and an ELISA-based assay for defensins, to determine their usefulness as early predictors of systemic neonatal infection.

Grant: 1R03HD039365-01
Program Director: KAUFMAN, STEVEN
Principal Investigator: STAPLETON, ANN E MD
Title: IN VITRO MODEL OF HUMAN VAGINAL EPITHELIUM
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2000/09/30-2003/08/31

DESCRIPTION (Adapted from applicant's description): The human vaginal epithelium is located in a key anatomical location, between the external environment and protected sites in the upper genital tract, and forms a barrier to infecting pathogens. A healthy vaginal ecosystem is critical to reproductive health. Estrogen replacement therapy and many contraceptive products and devices are vaginally delivered and may affect the vaginal epithelium. Despite the importance of the vaginal epithelium in reproductive health and disease, vaginal physiology and factors affecting differentiation of the vaginal epithelium are infrequently studied, and in vitro models of this tissue have not been widely developed. The applicant has been involved since 1995 in HD33202, a clinical study that defines unexplored parameters of normal vaginal physiology, defines changes induced by common vaginal products, and investigates these products effects on susceptibility to sexually transmitted diseases. As an outgrowth of this study, the applicant has developed a model of primary cultured vaginal cells (VECs) and has performed pilot studies of bacterial adherence and the effects of exogenous estrogen on cell growth and differentiation. Her preliminary data indicate that susceptibility of cultured VEC to bacterial adherence varies with (a) morphological parameters of differentiation; (b) the expression of glycosphingolipids (GSLs), which serve as differentiation markers and bacterial binding sites; and (c) dose and timing of exogenous estrogen exposure. The goal of this Small Grant proposal is to develop this promising model for basic investigations of epithelial differentiation and susceptibility to bacterial infection, before and after in vitro exposure to hormonal and vaginal products. The applicant hypothesizes that differentiation of vaginal epithelium affects its susceptibility to infection and its response to the application of exogenous hormones and contraceptive products. The applicant will pursue the following aims: (1) Primary VECs will be grown in culture and will be further characterized for expression of differentiation markers, such as keratins, epithelial differentiation markers extensively studied in related epithelia, and selected GSLs known to be involved in bacterial adherence. Vaginal tissue sections from normal young women, collected as part of HD33202, will be stained for the same characteristics; (2) Exogenous estrogen will be applied to VECs grown in culture and parameters of epithelial differentiation, such as the expression of keratins and of GSLs, will be investigated; (3) Studies of the adherence of Lactobacilli and of bacterial pathogens such as E. coli, which may relate to risk of premature birth, will be expanded to include the relationship between expression of differentiation markers and the effect of exogenous estrogen on susceptibility to bacterial attachment. Establishing this model will open opportunities for studying numerous aspects of women's urogenital health, including testing vaginal products, probiotics, and contraceptives; understanding protective roles of organisms in the normal flora; and studying the cellular effects of hormone replacement therapy on a key target tissue, the vagina.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1U01HD039165-01
Program Director: WILLOUGHBY, ANNE
Principal Investigator: CALDERWOOD, STEPHEN B MD INTERNAL
MED:INFECTIOUS DISEASE
Title: IMMUNE RESPONSES TO V. CHOLERAE INFECTION IN BANGLADESH
Institution: MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA
Project Period: 2000/09/05-2005/08/31

DESCRIPTION (adapted from application abstract): *Vibrio cholerae* causes a severe, dehydrating, and occasionally fatal diarrhea in humans. There are an estimated 5-7 million cases worldwide of cholera, with more than 100,000 deaths. Much of the impact of cholera occurs in developing areas of the world, particularly in South and Southeast Asia such as Bangladesh and India. Infection with *V. cholerae* induces long-lasting protective immunity to subsequent cholera, although the immune responses mediating protection are not fully understood. Many of the previous field studies of immune responses to *V. cholerae* infection were done in the 1970s, prior to the advent of more modern techniques for measuring mucosal immune responses, such as the use of antibody-secreting cell assays. More recent studies of *V. cholerae* infection in normal volunteers, many done in the United States, have provided important information on immune responses to infection with this pathogen, but these responses may differ substantially than those in patients in endemic areas, particularly as relates to the influence of age, morbidity, malnutrition and prior exposure to related antigens. Much work has been done recently on development of effective live, oral, attenuated *V. cholerae* vaccines, both for prevention of clinical cholera and as vectors for expressing heterologous antigens to protect against other infections at mucosal surfaces. This proposal would establish a long-term collaboration between scientists in the US and at the International Centre for Diarrhoeal Disease Research in Bangladesh to elucidate immune responses and protection from cholera infection in an endemic population. The Long-Term Goals of this project are to better understand mucosal immune responses after *V. cholerae* infection and vaccination, and to assess the effect of patient and microbial factors on these responses that may explain differences observed between patients from endemic areas and normal human volunteers. The Specific Aims of the proposal are: 1) determine the full range of immune responses, particularly mucosal antibody responses, in patients with cholera in Bangladesh, comparing vibriocidal and mucosal antibody responses and stratifying these responses by patient and microbial characteristics. We will test the hypothesis that the serum vibriocidal response represents a surrogate marker for a mucosal response to a relevant antigen or antigens that is actually protective; 2) correlate mucosal anti-*V. cholerae* antibody levels on exposure to the organism with protection from subsequent clinical cholera. We will examine the hypothesis that pre-existing secretory immunity to LPS, CtxB, MSHA and/or TcpA at the time of colonization of *V. cholerae* protects against subsequent illness; 3) assess immune responses to CtxB at distant (non-intestinal) mucosal sites after clinical cholera, as a model for immune responses to heterologous antigens expressed by live, oral, attenuated *V. cholerae* vaccine vectors.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2R01HL031237-16
Program Director:
Principal Investigator: KUNKEL, STEVEN L PHD PARASITOLOGY, OTM
Title: MONOKINE GENE EXPRESSION/REGULATION IN LUNG INJURY
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 1984/01/01-2004/02/28

DESCRIPTION (Adapted from the applicant's abstract): This application will study the biology of IL-13 and two new CC chemokines, C10 and MDC in the pathogenesis of sepsis following cecal ligation and puncture (CLP) in mice. Cytokine networks are critical in the host response to peritonitis and other forms of sepsis. The major hypothesis is that during the evolution of sepsis, IL-13 and CC chemokines induced by IL-13 regulate the host response and protect the host from organ injury. This hypothesis will be explored in 4 specific aims using a murine model of cecal ligation and puncture (CLP) in mice. Aim 1 will investigate the expression and tissue distribution of IL-13. Aim 2 will investigate the role of IL-13 in the pathogenesis of sepsis using several different strategies to neutralize IL-13. Aim 3 will investigate the expression and role of MDC and C10 in sepsis using methods similar to those in Aims 1 and 2. Aim 4 will use transgenic mice to explore the role of STAT6 as a key signaling molecule in the pathogenesis of sepsis in CLP. These studies will provide new information about the key roles of IL-13 and CC chemokines in the pathogenesis of the systemic response induced by CLP in mice.

Grant: 2R01HL047300-08A1
Program Director:
Principal Investigator: ANDERSON, JAMES MORLEY
Title: INFECTION MECHANISMS WITH CARDIOVASCULAR PROSTHESES
Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH
Project Period: 1991/08/01-2005/03/31

DESCRIPTION (Adapted from Applicant's Abstract): The proposed studies are directed toward obtaining a better understanding of the mechanisms of infection of implanted cardiovascular prostheses. The hypothesis is that material surface interactions with flowing blood lead to alteration of basic pathophysiologic mechanisms, which increase the probability of bacterial interaction and infection. The studies emphasize the use of clinically derived human materials, i.e., blood and bacteria, and clinically relevant cardiovascular materials coupled with controlled in vitro and in vivo systems to systematically and comprehensively elucidate infection mechanisms with prostheses. The overall goals of the project are to: 1] determine and quantify specific mechanisms of bacterial adhesion, 2] determine and quantify shear dependent non-specific mechanisms of bacterial adhesion, 3] evaluate leukocyte (PMN and monocyte) adhesion on materials, as mediated by plasma proteins and complement activation, in the presence of suspended and reseeded *S. epidermidis* under dynamic flow conditions, 4] investigate bacteria/leukocyte/biomaterial interactions which alter leukocyte function and microbial killing, 5] design, prepare and characterize biomimetic materials with bacteria-resistant properties that will undergo surface-induced assembly on cardiovascular biomaterials, and 6] utilize a biomaterial infection model in rats to identify in vitro to in vivo correlations. The experimental approach utilizes the variable shear stress rotating disk system and a new laminar flow system to determine interactions important in human blood protein/platelet/leukocyte interactions with *S. epidermidis* and clinically relevant biomaterials and novel bacteria-resistant biomaterial coatings. Quantification of bacterial interactions will be accomplished using high-resolution fluorescence microscopy, confocal and atomic force microscopies. Shear-dependent specific and non-specific mechanisms of bacterial adhesion will be identified. Leukocyte and monocyte adhesion and activation on biomaterials in the presence of *S. epidermidis* under variable flow will be characterized and correlated with leukocyte receptor expression, cell activation markers and cell function assays. Novel biomaterial design will be based on oligosaccharide surfactant polymer coatings containing a polymeric backbone with two types of side chains: one to facilitate adsorption to biomaterial surfaces, the other to generate a bacterial resistant surface. The applicant will optimize both the adsorption characteristics of the coating and its bacterial resistance through modification of the side chains. The biomaterial infection model in rats will be used to test the validity of the in vitro models, as well as the efficacy of the biomimetic coatings

Grant: 2R01HL051630-06

Program Director:

Principal Investigator: ELLNER, JERROLD J MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC

Title: EXPRESSION OF TUBERCULOSIS IN THE LUNG

Institution: UNIV OF MED/DENT NJ NEWARK NEWARK, NJ

Project Period: 1993/09/30-2005/08/31

DESCRIPTION (Adapted from the Applicant's Abstract): Mycobacterium tuberculosis infects a third of the worlds' population and TB is the leading cause of morbidity and mortality due a single infectious agent. However, only 5-10% of M. tuberculosis-infected subjects without an underlying immunodeficiency develop disease during their lifetimes. Therefore protective immunity is induced in the majority of subjects. Understanding correlates of protection against M. tuberculosis in humans is needed to better direct efforts in the development of antituberculosis vaccines. The PI suggests that increased susceptibility to M. tuberculosis infection by patients with IFNgamma receptor I deficiency and successful therapeutic use of IFNgamma in refractory mycobacterial infections indicates the importance of IFNgamma in immunity against mycobacteria. However, during the previous funding period the PI found increased levels of IFNgamma in the lungs of patients with TB. Thus, the question is raised as to the effectiveness of IFNgamma and/or involvement of other factors in protective immunity. In the present competitive renewal application, the PI proposes to define pulmonary correlates of protective immunity by comparing several immunological parameters in TB patients, healthy household contacts of TB patients (tuberculin skin test positive), and community control subjects. Bronchoalveolar and blood mononuclear cells obtained from each study subject will be utilized to characterize antigen-specific cytokine induction, killing of M. tuberculosis, CTL activity against M. tuberculosis-infected targets and mediators involved in these effector functions (iNOS, granzyme, perforin, granulysin, FasL) (specific aim 1). According to the PI, this first part of the work should identify correlates of protective immunity as differences between protective immune responses (in skin-test-positive, healthy household contacts) and failed immune responses (patients with TB). Correlates of protection identified in aim #1 should then be used to assess induction of protective immunity and chemokine expression by vaccination of humans with BCG. Two strains of BCG having different efficacy and reactogenicity, and two routes of vaccine administration (oral and intracutaneous) will be compared (specific aim 2). The PI states that this proposal attempts to define new parameters of immunological protection in humans and to rationally assess the impact of BCG strain variation and the route of BCG administration on the induction of protective immunity.

Grant: 2R01HL056036-04
Program Director:
Principal Investigator: KUO, CHO-CHOU
Title: ROLE OF CHLAMYDIA PNEUMONIAE INFECTION IN ATHEROGENESIS
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 1997/04/01-2004/03/31

DESCRIPTION (Adapted from the Applicant's Abstract): Chlamydia pneumoniae (TWAR) is a common human respiratory pathogen. In recent years, there has been mounting evidence showing that this organism might play a role in atherosclerosis. Because coronary heart disease is a leading cause of death in this country, the overall goal is to investigate the immunopathogenic mechanisms by which *C. pneumoniae* infection contributes to the development of vascular disease. The proposed studies will exploit our recent findings from mouse model studies linking *C. pneumoniae* infection and atherosclerosis and in vitro cell culture studies on *C. pneumoniae* infection of arterial wall cells. The mouse models that will be used are C57BU6 and strains derived from this background strain including, apoE-deficient and TNF-A receptor and apoE double knockout mice. Atherosclerosis in C57BU6 mice can be induced by feeding with a high fat/high cholesterol diet, while apoE mice develop atherosclerosis spontaneously on a regular diet. The specific aims are to 1) further evaluate the synergistic effect of *C. pneumoniae* infection and hyperlipidemia on atherogenesis by infecting mice with *C. pneumoniae* followed by feeding animals with a high fat/high cholesterol diet and measuring the atherosclerotic lesion development using computer assisted morphometry; 2) study the effects of *C. pneumoniae* infection on key components in the inflammatory process of atherosclerosis that promote atherosclerotic lesion development by recruiting lymphocytes/macrophages and eliciting inflammatory responses at lesion sites. In vitro, in vivo, and ex vivo systems will be used to assay the expression of leukocyte adhesion molecules and adherence of macrophages to the endothelial surface. The effect of TNF-A on lesion development will be investigated by infecting TNF-A receptor and apoE double knockout mice and measuring lesion development using computer assisted morphometry; 3) assess the role of macrophages in the establishment of persistent *C. pneumoniae* infection of atheromatous lesions using cell culture to analyze vascular cell interactions and the effect on infectivity, growth and persistence of *C. pneumoniae*, and characterize the growth of *C. pneumoniae* in macrophages loaded with low density lipoproteins (foam cells). The proposed studies should prove invaluable for understanding the disease process and developing better measures for eradication or prevention of *C. pneumoniae* infection and for reducing atherosclerosis and coronary heart disease.

Grant: 2R01HL058897-04
Program Director:
Principal Investigator: PETERS-GOLDEN, MARC L
Title: EICOSANOIDS AND LUNG MACROPHAGE ANTIMICROBIAL MECHANISMS
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 1997/07/10-2004/06/30

DESCRIPTION (adapted from application): Modulating innate pulmonary defense mechanisms for therapeutic advantage requires a better understanding of the molecules that mediate the antimicrobial actions of phagocytic cells. Although most research in this arena has focused on peptide mediators, or laboratory has recently identified an important and heretofore unrecognized role in antimicrobial defense for an alternative group of mediators, namely, leukotrienes (LTs) and other metabolites of the 5-lipoxygenase (5-LO) pathway of arachidonate metabolism. The investigators' work has demonstrated that LTs are produced by phagocytes in the context of bacterial infection, that LTs are required for optimal bacterial clearance from the lung, and that LTs enhance the capacity of alveolar macrophages (AMs) for bacterial phagocytosis and killing. Despite this progress, we have an incomplete understanding of the molecular mechanisms by which microbes trigger endogenous LT synthesis during their encounter with phagocytes, and by which LTs augment microbial phagocytosis and killing. Moreover, preliminary data suggest that other arachidonate metabolites (eicosanoids), including prostaglandin E2 (PGE2) and 15-hydroxyeicosatetraenoic acid (15-HETE), suppress antimicrobial activity of AMs. The central hypothesis of the present proposal is that the effects of eicosanoids on AM phagocytosis and killing reflect the amplification (in the case of LTs) or down-regulation (in the case of PGE2 and 15-HETE) or molecular signals generated upon cellular interaction with immunoglobulin-opsonized phagocytic targets. This hypothesis will be tested in a series of in vitro experiments utilizing cultured AMs challenged with either IgG-coated erythrocytes or bacteria. The aims are to: 1) determine the enzymatic pathways that mediate arachidonate release and metabolism triggered by ligation of the AM receptor for the Fc portion of IgG (FcR), and the post-receptor signal transduction events that are responsible for eicosanoid generation; 2) identify the signal transduction events amplified by LTs that result in augmented FcR-dependent phagocytosis and killing; and 3) determine the relative importance of inhibition of LT synthesis vs. inhibition of FcR-dependent signaling in the ability of PGE2 and 15-HETE to down-regulate phagocytosis and killing by AMs. These studies will provide critical mechanistic information about an understudied and clinically relevant arm of the innate host defense system.

Grant: 1R01HL062356-01A2

Program Director:

Principal Investigator: HAWIGER, JACK J MD INTERNAL
MED:HEMATOLOGY

Title: MOLECULAR MECHANISMS OF SEPTIC SHOCK

Institution: VANDERBILT UNIVERSITY NASHVILLE, TN

Project Period: 2000/08/01-2004/07/31

The focus of this application is molecular mechanisms of septic shock with attendant Disseminated Intravascular Coagulation (DIC) affecting annually 400,000 hospitalized patients in the US. Septic shock along with acute respiratory distress syndrome and multiple organ failure constitutes the extreme form of systemic inflammatory response syndrome. The prototypical inducers of septic shock are endotoxin (lipopolysaccharide, LPS), a product of Gram-negative bacteria and superantigens, a product of Gram-positive bacteria. In many cases these bacterial infections occur in the setting of indwelling catheters coated with bacterial biofilms following their insertion for neonatal parenteral nutrition, accident- and surgery-associated trauma, cancer chemotherapy, and immunosuppression for bone marrow and other transplants. The central and unifying hypothesis of the molecular mechanism of septic shock is based on intracellular signaling to the nucleus in response to inflammatory, oxidant, and immune stress. This signaling is mediated by transcription factors NF-kappaB, AP-1, and others controlling expression of multiple genes encoding cytokines and chemokines, procoagulant and cell adhesion molecules, inducible NO synthase and cyclooxygenase 2, and apoptosis regulators. Activation of NF-kappaB requires signal-dependent phosphorylation and intracellular proteolysis of inhibitory molecules, I-kappaB α , and epsilon, sequestering NF-kappaB in cytoplasm. The key signal transducers responding to inducers (LPS) and mediators (cytokines) of septic shock are recently discovered I-kappaB kinase (IKK) complex. To establish their pivotal role in the molecular mechanism of cellular responses to LPS and toxic superantigens we will analyze regulation of I-kappaB kinases in monocytic and T cells targeted by LPS and superantigens. The first level of analysis will involve the role of Toll-like receptors, in activation of IKK complex by LPS. The second level of analysis will be activation of IKK complex by superantigen-T cell receptor CD3 signalling. The third level of analysis will be signaling to the nucleus by NF-kappaB and other transcription factors based on Nuclear Localization Sequence (NLS) recognition by importins/karyopherins involved in nuclear import of transcription factors. Novel technologies of non-invasive delivery of peptides, protein and recombinant fragments to cells involved in septic shock will be developed and tested in vitro and in vivo to attenuate activation of genes regulated by NF-kappaB and other transactivators to prevent monocytic, endothelial and T cells from acquiring a "septic shock activated" phenotype. Implicit in this analysis of the molecular mechanism of septic shock and DIC is the development of new therapies targeted at intracellular signaling to the nucleus. The ultimate goal of the proposed studies is to provide a new conceptual framework for reduction of high mortality due to septic shock.

Grant: 1R01HL062400-01A1

Program Director:

Principal Investigator: BAXTER, B TIMOTHY MD OTHER AREAS

Title: MMP REGULATION BY DOXYCYCLINE IN AORTIC ANEURYSM

Institution: UNIVERSITY OF NEBRASKA MEDICAL OMAHA, NE
CENTER

Project Period: 2000/02/01-2005/01/31

Abdominal Aortic Aneurysm (AAA) is a common and devastating disease which is increasing in incidence. Although easy and inexpensive to detect by ultrasound, most aneurysms are small when detected and there is currently no medical regimen which will inhibit their growth. There is an increasing body of evidence implicating a family of matrix degrading enzymes, the matrix metalloproteinases (MMPs) in AAA. Although both MMP-9 and MMP-12 may have a role in AAA, we have identified a significant increase in total MMP-2 in AAA. Importantly, a larger proportion of the MMP-2 in AAA tissue is in the active form and is directly bound to the matrix suggesting ongoing proteolysis. In addition, we have demonstrated that AAA tissue contains increased levels of membrane type 1 MMP, the activator of MMP-2. We have also shown that doxycycline inhibits MMP-2 production by aortic smooth muscle cells in culture. We hypothesize that MMP-2, through its increased activation, has a central role in aneurysm formation and that this could be inhibited by doxycycline. This hypothesis will be examined through the following specific aims: 1. Determine the effects of individual MMPs implicated in AAA including MMP-2, MT1-MMP, MMP-9 and MMP-12 on the size and rate of aneurysm formation in a murine AAA model. 2. Determine the effects of doxycycline on the size and rate of aneurysm formation and progression in a murine model and correlate these effects with serum doxycycline levels. 3. Determine the mechanisms by which doxycycline down regulates MMPs in human aortic smooth muscle cells. Specific aim 1 will be accomplished using a mouse model of AAA characterized in our laboratory with four different knock-out mice, including MMP-2, MMP-9, MMP-12 and a TIMP-2 knock-out mouse in which activation of MMP-2 does not occur. Specific aim 2 will be accomplished by using doxycycline treatment in our murine model of AAA and correlating effects on aortic MMP expression, aneurysm size and growth rate with serum doxycycline concentrations. Specific aim 3 will be accomplished by determining MMP-2 mRNA levels, mRNA half life, rate of mRNA transcription and identifying the doxycycline responsive elements in the MMP-2 promoter. The long term goal of this work is to develop pharmacologic therapies which specifically target MMPs important in aneurysm pathogenesis and progression.

Grant: 1R01HL064038-01
Program Director:
Principal Investigator: GILLES-GONZALEZ, MARIE A PHD
Title: MUTAGENESIS OF FIXL, AN O₂ SENSING PAS DOMAIN PROTEIN
Institution: OHIO STATE UNIVERSITY COLUMBUS, OH
Project Period: 2000/02/07-2003/12/31

The FixL protein is a biological sensor of O₂. Dissociation of O₂ from a heme-binding domain is required for FixL to autophosphorylate at a conserved histidine with a gamma-phosphoryl group from ATP. Transfer of the phosphoryl group from FixL to the transcription factor FixJ enables FixJ to activate a cascade of gene expression. The kinase domain of FixL belongs to a large class of sensory histidine kinases that occur in plants, fungi, Archaea, and every bacterium that has been examined. The structure of such a kinase domain has recently become available. Crystal structures were also recently solved for the heme-binding domain of FixL with and without an inhibitory ligand. These FixL structures have led to a new model for heme-driven conformational changes. Although the alpha/beta fold of the FixL, heme-binding domain represents a novel hemoglobin fold, this fold closely matches the structures of the HERG voltage sensory in humans and the PYP light sensory in halophilic bacterium. As such, the heme-binding domain of FixL provides a prototype for a large family of sensory proteins called the PAS proteins. Based on sequence alignments, over 200 PAS proteins have been identified so far in Archaea, Bacteria, and Eukarya, where they transduce key regulatory signals. The direct coupling in FixL of a histidine kinase to an O₂-detecting PAS domain provides an ideal system for study of signal transduction. The proposed work will combined mutagenesis with biochemical, biophysical, and structural approaches to: test the regulatory mechanism indicated by the FixL "on" and "off" structures, examine the role of conserved PAS residues, determine factors that govern affinity for ligands, and identify key contacts between the heme-binding and kinase domains of FixL. This research is expected to extend knowledge of signal transduction mechanisms and sensing of physiological heme ligands. Some possible applications are the development of novel antibiotics directed at specific sensors in bacteria and anti-cancer drugs targeting O₂ sensors that are implicated in angiogenesis.

Grant: 1R01HL065397-01
Program Director:
Principal Investigator: JOBE, ALAN H MD OTHER AREAS
Title: NEW MEDIATORS OF CLINICAL LUNG MATURATION
Institution: CHILDREN'S HOSPITAL MED CTR CINCINNATI, OH
(CINCINNATI)
Project Period: 2000/09/01-2004/08/31

DESCRIPTION: (Adapted from the Investigator's Abstract): Fetal sheep will be exposed to intraamniotic endotoxin and the minimal effective dose determined as well as the effects of prolonged low dose exposure. Subsequently, the route of the effects of endotoxin will be evaluated by targeting the stimulus to the amniotic cavity, the systemic circulation, the GI tract, or the lungs. All lambs exposed in utero will be delivered preterm and evaluated for indicators of amniotic space inflammation, lung function, and lung biochemical indicators of maturation. Candidate mediators, identified by evaluating the fetal responses to endotoxin, will be tested for their ability to induce fetal responses and lung maturation.

Grant: 1R01HL065752-01
Program Director:
Principal Investigator: LOOK, DWIGHT C MD
Title: EPITHELIAL CELL RESPONSE TO H.INFLUENZAE IN THE AIRWAY
Institution: WASHINGTON UNIVERSITY ST LOUIS, MO
Project Period: 2000/09/20-2001/07/31

DESCRIPTION (adapted from the application): Inflammation of the airway epithelium is often required for effective innate defense against microbes, and epithelial cells provide critical biochemical signals that regulate this response. One major mechanism that epithelial cells in the airway use to participate in the inflammatory response is through regulation of leukocyte trafficking and/or activation by expression of intercellular adhesion molecule-1 (ICAM-1). ICAM-1 serves as a ligand for leukocyte beta2-integrins and thereby mediates epithelial-leukocyte interactions that may allow for "appropriate" inflammatory responses (e.g., to a respiratory bacterial infection) or "inappropriate" responses (e.g., airway inflammation in cystic fibrosis). This proposal focuses on *Haemophilus influenzae*, which frequently colonizes human respiratory mucosa and often produces respiratory tract disease, particularly in patients with chronic bronchitis, bronchiectasis, and cystic fibrosis. The specific aims of this proposal are based on four observations regarding airway epithelial cell ICAM-1 expression in response to *H. influenzae*: 1) *H. influenzae* induces airway epithelial cell ICAM-1 expression in vivo and in vitro; 2) ICAM-1 expression is required for efficient bacterial clearance in a murine model of airway infection with *H. influenzae*; 3) increased ICAM-1 expression can be initiated by epithelial cell interaction with a constitutive molecule on the bacterial cell surface; and 4) airway epithelial cell interaction with *H. influenzae* results in generation of soluble ICAM-1 inducing activity containing a novel mediator(s) of ICAM-1 expression. Based on these observations, they hypothesize that direct induction of specific epithelial genes (such as ICAM-1) allow for rapid targeting and/or activation of neutrophils and other leukocytes at sites of *H. influenzae* infection, resulting in efficient innate defense in the airway. Accordingly, there are two specific aims. 1) Define mechanisms for induction of epithelial cell ICAM-1 expression by *H. influenzae*. This aim will take advantage of in vitro coculture models of epithelial cell interaction with bacteria. Definition of mechanisms for ICAM-1 gene activation in response to *H. influenzae* will be accomplished by analysis of ICAM-1 promoter function and identification of mediator molecules. This latter refers to the observation that airway epithelial cells challenged with *H. influenzae* release a novel soluble factor into the medium capable of eliciting ICAM-1 in naive epithelial cells. 2) Determine functions of ICAM-1 in defense against *H. influenzae* infection. This aim will take advantage of in vivo murine models of airway infection by bacteria. The functions of ICAM-1 will be determined by examining ICAM-1 expression, leukocyte recruitment and function, and bacteria clearance under conditions that allow for manipulation of airway defense factors.

Grant: 1R01HL065898-01

Program Director:

Principal Investigator: WILSON, CHRISTOPHER B MD OTHER CL
MED:CLINICAL
MEDICINE,UNSPEC

Title: REGULATION OF PSEUDOMONAS INDUCED LUNG INFLAMMATION

Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA

Project Period: 2000/04/01-2004/03/31

The mechanisms by which lung inflammation is initiated and perpetuated in response to infection are incompletely understood. Pro-inflammatory cytokines, chemokines and adhesion molecules contribute, as do alveolar macrophages and respiratory epithelial cells which produce them, but their relative importance may differ depending on the host and the nature of the infection. In patients with cystic fibrosis (CF), lung inflammation commonly develops in early infancy and then progresses, particularly following acquisition of infection with *Pseudomonas aeruginosa*. How the defect in CHR expression results in the intense and progressive lung inflammatory response and predisposition to refractory infection with *P. aeruginosa* is unclear. This lack of understanding parallels a paucity of information regarding the role which the respiratory epithelium plays in the regulation of lung inflammation in general. This reflects the absence heretofore of a selective and robust approach by which to test the contribution of the respiratory epithelium. Similarly, an important role for TNF in lung inflammation in response to infection with *P. aeruginosa* and in CF has been proposed based on correlative human data and results in some rodent models, but the latter studies have yielded contradictory results. This proposal addresses the general hypothesis that TNF acts in concert with the respiratory epithelium to regulate lung inflammation and innate immunity to *Pseudomonas aeruginosa*, and that aberrant regulation leads to excess lung inflammation in CF. Aim 1) Explore the basis for the increased early lung inflammatory response to *P. aeruginosa* in TNF receptor-deficient mice. Hypothesis: The selective early increase in neutrophil recruitment and bacterial clearance following acute aerosol infection with *P. aeruginosa* will be due to an altered inflammatory response by lung parenchymal cells; this will reflect, at least in part, altered expression by these cells of microbial pattern recognition receptors that transduce inflammatory signals in response to *P. aeruginosa*. Aim 2) Explore the role of the airway epithelium in the pulmonary inflammatory response to *P. aeruginosa* using mice in which NF-kappaB activation is blocked selectively and in a cell-autonomous fashion in the airway epithelium. Hypothesis: The airway epithelium will play an important role in initiating acute lung inflammation in response to *P. aeruginosa*. Aim 3) Determine the degree to which lung inflammation is increased in the lungs of CFTR knockout mice, and if so, if this is intrinsic to the lung and due in part to aberrant activation of NF-kappaB in the airway epithelium. Hypothesis: CFTR KO mice will have excessive lung inflammation. This will reflect a process intrinsic to the lung and will parallel and be dependent, at least in part, on NF-kappaB activation in the airway epithelium.

Grant: 1R01HL066548-01

Program Director:

Principal Investigator: REMOLD-O'DONNELL, EILEEN PHD

Title: ROLE OF PROTEASE IN INFECTION & INFLAMMATION OF COPD

Institution: CBR INSTITUTE FOR BIOMEDICAL RESEARCH BOSTON, MA

Project Period: 2000/09/29-2004/08/31

DESCRIPTION (adapted from the applicants' abstract) The project will analyze the role of the neutrophil/monocyte serine proteases, elastase, cathepsin-G and proteinase-3, and their specific inhibitors, alpha-1-antitrypsin and especially MNEI (monocyte/neutrophil elastase inhibitor), in infection and inflammation of COPD. The hypothesis to be evaluated is that excess of the proteases in inflammatory lung fluids contributes to disease progression, not only by inducing inflammation and injury, but also importantly by targeting and destroying innate anti-microbial pulmonary defense molecules. Based largely on the investigators recent findings, the investigators hypothesize that an important innate defense molecule targeted by the proteases, and protected by MNEI, is surfactant protein-A (SP-A), an opsonin and activating agent, known to enhance uptake and clearance of microbes by resident pulmonary macrophages. Studies are proposed in a mouse model of excess neutrophil/monocyte protease activity, which the investigators will generate by targeting the mnei gene. Mnei-A def deficient mice will be compared to wildtype mice for their ability to clear pulmonary infections with *Streptococcus pneumoniae* and nontypeable *Haemophilus influenzae*, significant infectious agents in chronic bronchitis. Inflammatory parameters (neutrophil influx, free protease, SP-A levels, cytokines, chemokines) will also be compared. Using a complementary human in vitro system, the investigators will compare inflammatory lung fluids (BAL supernatants) of COPD patients with lung fluids of normal healthy non-smokers and smokers, for their ability to enhance uptake of *S. pneumoniae* and *H. influenzae* by normal macrophages. The investigators will examine also the effect of exogenous protease on bacterial uptake enhancing activity (including SP-A). Clarifying the role of elastase, cathepsin-G and proteinase-3 in disease progression of COPD is important, among other reasons, because therapeutic modalities are now feasible and could be delivered to the patients if the underlying pathological processes were understood.

Grant: 1R01HL066549-01
Program Director:
Principal Investigator: SETHI, SANJAY MD
Title: HAEMOPHILUS ANTIGEN REGULATION OF INFLAMMATION IN COPD
Institution: STATE UNIVERSITY OF NEW YORK AT AMHERST, NY
BUFFALO
Project Period: 2000/09/29-2004/08/31

DESCRIPTION (adapted from the applicants' abstract) The contribution of airway inflammation to the pathogenesis of COPD has received increasing recognition. Nontypeable Haemophilus influenzae (NTHI) is the most common bacterial pathogen in the lungs of patients with COPD, and is present in all stages of this disease. NTHI and its components have numerous proinflammatory effects on respiratory tissues. Nonetheless, the role of NTHI as an independent stimulus to inflammation in COPD has not been explored. Alveolar macrophages are critical to host interactions with NTHI. Furthermore, alveolar macrophages respond to inflammatory stimuli distinctly unlike macrophages of extra-pulmonary tissues. Yet interactions of alveolar macrophages with NTHI, and the impact on pathogenesis of COPD, remains unknown. The investigators propose studies designed to gain broader insight into the immunologic mechanisms by which NTHI mediates inflammation in COPD. For all studies, three groups of subjects will be recruited: a) former smokers with COPD; b) former smokers without COPD; and c) healthy non-smokers. In Specific Aim 1, the association between NTHI 'colonization' and levels of inflammatory cells and mediators, in the airway lumen and in tissues, will be determined. Inflammatory cytokines and elastase will be measured in bronchoalveolar lavage (BAL) fluid and inflammatory cells will be enumerated in BAL and in bronchial submucosa. NTHI in the BAL and bronchial biopsies will be detected by culture, PCR and by immunostaining. In Specific Aim 2, the biological effects of outer membrane antigens of NTHI on alveolar macrophage function will be determined. Induction of regulatory cytokines, adhesion molecules and functional capabilities of alveolar macrophages by immunologically active NTHI antigens will be evaluated. Inflammatory and functional parameters will be compared with responses of same-host blood-derived mononuclear phagocytes. Identification of the integral bacterial and host components that contribute to immune-mediated inflammation in COPD will ultimately lead to novel therapeutic strategies to alter disease progression and improve outcome in COPD.

Grant: 1R01HL066559-01
Program Director:
Principal Investigator: PEDEN, DAVID B MD
Title: CD14 AND LPS-INDUCED INFLAMMATION IN CHRONIC BRONCHITIS
Institution: UNIVERSITY OF NORTH CAROLINA CHAPEL HILL, NC
HILL
Project Period: 2000/09/29-2004/08/31

DESCRIPTION (adapted from the applicants' abstract) Chronic bronchitis and COPD are characterized by chronic neutrophilic inflammation and is associated with both airway sepsis with gram-negative bacteria. Smoking is a major risk factor for development of these diseases, but only about 20 percent of smokers develop COPD. Endotoxin (ET) from gram-negative bacteria likely plays a significant role in this inflammation. Airway ET may come directly from gram-negative bacteria infecting the airway or from tobacco smoke as this is another rich source of ET. The investigators propose that responsiveness to ET also an important risk factor for development of COPD in smokers. One mechanism by which persons may be more sensitive to ET is by enhanced production of CD14, the primary receptor for ET. CD14 exists in both a cell bound form and as a soluble form in serum and airway secretions. Soluble CD14 in the airway is enhanced by acute allergic inflammation. Additionally, a C/T polymorphism has been identified at the -159 position of the CD14 promoter gene (CD14 gene). Those persons homozygous for the T allele have been shown to have increased soluble CD14 in serum and CD14 expression on blood monocytes compared to those with CT or CC genotype. The investigators present preliminary data that demonstrates that levels of sCD14 in sputum and CD14 expression on alveolar macrophages prior to challenge with lipopolysaccharide (LPS, a form of ET) correlates with neutrophil influx in sputum following inhaled LPS challenge. Also, in the nasal airway, allergen enhances granulocyte response to LPS in a fashion which correlates with local sCD14 levels. The investigators propose to examine the role that CD14 has in determining responsiveness to airway LPS and risk for COPD in smokers. First, the investigators will determine if the level of sCD14 and CD14 on macrophages is increased in volunteers with experimentally-induced and naturally occurring bronchitis and if they have increased neutrophil response to LPS. Second, the investigators determine if airway CD14 correlates with PMN response to LPS in normal volunteers. Third, the investigators will determine if airway CD14 levels and LPS response in healthy volunteers with the TT genotype for the CD14 gene is enhanced relative to that in those with the CC and CT genotype. Finally, the investigators will genotype cohorts of COPD patients and healthy smokers to determine if the T allele is a risk factor in development of COPD in those who smoke.

Grant: 1U01HL066952-01
Program Director:
Principal Investigator: CRYSTAL, RONALD G
Title: WEILL CORNELL PROGRAM OF EXCELLENCE IN GENE THERAPY
Institution: WEILL MEDICAL COLLEGE OF CORNELL NEW YORK, NY
UNIV
Project Period: 2000/09/28-2005/08/31

The unifying theme of the Weill Cornell PEGT is the challenge of adapting the technology of ex vivo and in vivo gene transfer to treat and prevent disorders of heart, lung and blood. This challenge can be met by understanding the biology of gene transfer to cells and experimental animals and applying that understanding to the design of clinical studies. The Weill Cornell PEGT combines extensive gene therapy core facilities with 6 NIH funded Principal Investigators at Weill Cornell, Memorial-Sloan Kettering, and Evanston Northwestern, with overlapping interests and ongoing collaborations including 2 NIH Program Projects and 5 shared R01 grants. The proposed PEGT comprises 4 pre-clinical projects, 2 clinical projects, 8 cores, and a data management program. The projects include: (1) Genetic Treatment of - thalassemia by lentivirus-mediated transfer of a regulated human-globin gene (M. Sadelain), (2) In vivo expansion, mobilization and recovery of bone marrow-derived stem cells by regional delivery of adenoviral vectors expressing cytokines (S. Rafii), (3) Manipulation of hematopoietic and endothelial stem cell self-renewal and proliferation by adeno- and retroviral gene transfer (M. Moore); (4) Development of a anti-Pseudomonas vaccine using dendritic cells modified to express CD40L and pulsed with Pseudomonas (R. Crystal); (5) retroviral mediated transfer of the glucose-6-phosphate dehydrogenase gene into human hematopoietic progenitor cells for the treatment of patients with chronic non-spherocytic hemolytic anemia (L. Luzzato), myocardial angiogenesis therapy as an adjunct to off-pump coronary artery bypass surgery (T. Rosengart). The supporting cores include: DNA vector, RNA vector; Stem cell; Analysis; Clinical Operations and Regulatory Affairs; Experimental Animal; Training and Education; Administration; and PEGT Data Management.

Grant: 2P01MH049351-07
Program Director: BRADY, LINDA S.
Principal Investigator: LECKMAN, JAMES F MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC
Title: PATHOGENESIS & TREATMENT OF TS, OCD & RELATED DISORDERS
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 1992/09/30-2003/08/31

DESCRIPTION (Adapted from applicant's abstract): This program project focuses on the pathogenesis of Tourette's syndrome (TS) and obsessive-compulsive disorder (OCD) as models for understanding the interplay of genetic, neurobiological, and psychological factors during the course of CNS development. TS, OCD, and related conditions are prevalent disorders affecting as many as 0.3-3% of the population. They are frequently chronic and associated with marked impairment and disability. Although clinical care has improved over the past decade, a significant number of patients fail to respond adequately or experience intolerable side effects. The etiology of these disorders is unknown. Compelling evidence suggests that the vulnerability to develop TS and OCD is mediated by both genetic and environmental factors, and that neural systems located in the basal ganglia and functionally related brain structures are involved in their pathogenesis. Based on explicit models of pathogenesis for TS and OCD and building on work accomplished over the past six years, an array of clinical, neuropsychological, genetic, neuroimaging, epidemiological, neurobiological, and treatment studies are proposed. Translational and integrative studies of perinatal and immunological risk factors are of particular significance. If confirmed as being causally related to TS and OCD, they may lead to clinical and public health interventions, as well as the development of valid animal models. A multidisciplinary team of investigators has joined forces to test specific hypotheses through the integration and translation of basic and clinical neuroscience research. All subjects will be studied using identical clinical, neuropsychological, genetic, and neurobiological techniques. These studies are divided into seven projects and are supported by three cores.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 1R01MH058868-01A2
Program Director: STOFF, DAVID M.
Principal Investigator: MC MAHON, WILLIAM M MD
Title: STREP THROAT, SYDENHAM CHOREA, AND TOURETTE SYNDROME
Institution: UNIVERSITY OF UTAH SALT LAKE CITY, UT
Project Period: 2000/03/01-2005/02/28

Abstract Text Not Available

Grant: 1P01NS039546-01A1
Program Director: NICHOLS, PAUL L
Principal Investigator: CASSONE, VINCENT M PHD
Title: COORDINATION OF CIRCADIAN PHYSIOLOGY OF DIVERSE SPECIES
Institution: TEXAS A&M UNIVERSITY SYSTEM COLLEGE STATION, TX
Project Period: 2000/07/01-2005/06/30

The biological clocks that regulator daily and circadian patterns of daily behavior, physiology and biochemistry are fundamental properties of biological organization. In recent years, great strides have been made in elucidating the anatomical localization of clock structures, the physiology of clock function and the biochemical/molecular events underlying circadian rhythmicity. These advances have been made in diverse organisms, which share many common formal and molecular properties but which differ in the level at which clocks are understood and at which they function. Among the best-studied model systems are the cyanobacterium *Synechococcus* sp., the filamentous fungus *Neurospora crassa*, the avian pineal gland and the mammalian suprachiasmatic nucleus (SCN), which have all provided disparate pieces of a very large, complex puzzle. However, a complete picture of this puzzle, of clock input, oscillation and output, is not apparent, because divergent systems provide dissimilar views. This may be due to real biological differences in experimental systems and/or diverse approaches. The present Program Project Grant combines the efforts of 4 established laboratories, which have a history of cooperative education and research, devoted to different aspects of biological clock function in these well-studied model systems. Dr. D.J Earnest (Project #2) will investigate mechanisms of circadian oscillation and its output in a unique immortalized cell line derived from the rat SCN. Dr. D. Bell-Pedersen (Project #3) will explore the relationship of developmental and metabolic processes with circadian rhythm function in *Neurospora*. Dr. V.M. Cassone (Project #4) will study the molecular mechanisms of rhythm generation and coupling to melatonin biosynthesis in the chick pineal and of cellular mechanisms of melatonin action in cultured glia. Two Technical Cores and an Administrative/Integrative Core will enable each laboratory to extend the boundaries of their own research: 1) The Genomics (Core A), directed by Dr. T. Thomas, will perform modern genomics analyses including high density transcriptional profiling, DNA microarray technology and EST analyses. 2) The Cell Physiology/Imaging Core (Core B), directed by Dr. Mark Zoran, will perform electrophysiological and real-time imaging of gene expression. 3) The Administrative and Integrative Core (Core C), directed by Dr. Cassone, will maintain records, facilitate interaction of laboratories by scheduling of regulate meetings, and coordinate meetings with advisory committees. In addition, this ore will facilitate data analysis of the entire data set collected by all laboratories compiling sequence and functional data, archiving these data and analyzing phylogenetic relationships among these diverse systems. All four projects are bonded by common technological approaches to diverse biological systems and by a commitment to interdisciplinary, comparative analyses of biological clock function.

Includes Research Project Grants (RPGs)
Excludes clinical trials

Grant: 2R01NS026310-13
Program Director: KERZA-KWIATECKI, A P
Principal Investigator: KIM, KWANG SIK MD
PEDIATRICS:PEDIATRICS-
UNSPEC
Title: E COLI INVASION OF BRAIN ENDOTHELIAL CELLS
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 1988/03/01-2004/08/31

Description (Adapted from the applicant's abstract): The mortality and morbidity associated with neonatal bacterial meningitis have remained significant despite advances in antimicrobial chemotherapy and supportive care. Inadequate knowledge of the pathogenesis and pathophysiology has contributed to this high mortality and morbidity. *E. coli* is the most common gram-negative organism that causes meningitis during the neonatal period. Most cases of *E. coli* meningitis in newborns develop as a result of hematogenous spread, but it is not clear how circulating *E. coli* cross the blood-brain barrier. We have established an infant rat model of experimental hematogenous meningitis which mimics human *E. coli* meningitis (e.g. hematogenous infection of meninges). We have also established an in vitro model of the blood-brain barrier with brain microvascular endothelial cells (BMEC). Using these in vitro and in vivo systems, we have shown that invasion of BMEC is a requirement for *E. coli* K1 crossing of the blood-brain barrier in vivo. During the previous funding period, we have shown that several *E. coli* K1 determinants contribute to invasion of BMEC in vitro and crossing of the blood-brain barrier in vivo (i.e., OmpA, IbeA, IbeB, IbeC, CNF1). We also showed that *E. coli* K1 invasion of endothelial cells is specific to BMEC, and no such invasion was observed for endothelial cells of non-brain origin. We have so far shown that some of the *E. coli* proteins (e.g., OmpA, IbeA) interact with specific receptors present on BMEC, not on systemic vascular endothelial cells. In addition, we showed that actin cytoskeleton rearrangements are involved in *E. coli* K1 invasion of BMEC, as shown by invasive *E. coli* K1-associated F-actin condensation and blockade of invasion by cytochalasin D. Based on the resources and findings derived from the past funding period, we would like to examine the following specific aims. 1. To continue to identify and characterize microbial determinants contributing to *E. coli* K1 invasion of BMEC in vitro and in vivo 2. To examine the mechanisms involved in *E. coli* K1 invasion of BMEC, including structure-function analysis of *E. coli* proteins, and identification and characterization of BMEC receptors. 3. To determine host cell signal transduction pathways involved in *E. coli* K1 invasion of BMEC, including focal adhesion kinase (FAK), Rho and phosphatidylinositol (PI)3-kinase. Further understanding and characterization of these *E. coli* K1-BMEC interactions should allow us to develop novel strategies to prevent this serious infection.

Grant: 2R01NS034857-04A1
Program Director: KITT, CHERYL A.
Principal Investigator: BLATTEIS, CLARK M
Title: PATHOPHYSIOLOGY OF ENDOTOXIN-MEDIATED FEVER
Institution: UNIVERSITY OF TENNESSEE HEALTH SCI MEMPHIS, TN
CTR
Project Period: 1996/09/01-2004/03/31

DESCRIPTION (adapted from applicant's abstract): It is generally believed that fever is caused not by exogenous, but by endogenous pyrogens, members of an array of peptide mediators called cytokines. These are elaborated and released into the circulation largely by systemic mononuclear phagocytes activated by the exogenous agents, and transported to the preoptic anterior hypothalamic area (POA) of the brain, where they act. Prostaglandin E2 (PGE2) is thought to be a fever mediator in the POA, induced by these cytokines. Although the intrapreoptic level of PGE2 rises rapidly following the intravenous (iv) administration of exogenous (e.g., lipopolysaccharide, LPS) or endogenous pyrogens, recent data indicate that these substances cannot account directly for the rapid production of PGE2 because it is initiated well before the synthase that they stimulate (cyclooxygenase-2, COX-2) becomes active. Moreover, cytokines also are not detectable in blood following i.v. LPS until after the febrile response is already underway, and there is no evidence that they can readily penetrate the brain. Hence, other, more quickly evoked mediators may be presumed to be involved in initiating the febrile response. Based on well-recognized mechanisms of LPS action, we have hypothesized that LPS virtually immediately activates the complement (C) cascade, generating the anaphylatoxins C3a and C5a, which function to activate macrophages and rapidly induce a mediator that has the capacity to stimulate peripheral sensory nerves that then transmit this information to the POA. We have data supporting the involvement of systemic C in the febrile response of guinea pigs to LPS, but more critical when LPS is injected i.p. than when it is administered iv. We hypothesize from preliminary data that this differential dependence on C may be related to distinct functional and biochemical characteristics of peritoneal and hepatic macrophages. Finally, recent preliminary data have suggested that the released mediator that acts on neural afferents may not be PGE2 and may also not be derived from macrophages, as had been supposed. The purpose of this proposed research, therefore, is to address the issues raised by our findings. The results will contribute to understanding the mechanisms of peripheral pyrogenic signaling, particularly regarding the onset of fever, as well as the processes that underlie the multivariate host defense reactions to infectious stimuli in general.

Grant: 1R01NS037467-01A3
Program Director: KERZA-KWIATECKI, A P
Principal Investigator: SRIRAM, SUBRAMANIAM MB
Title: ANALYSIS OF ASSOCIATION BETWEEN C. PNEUMONIAE AND MS
Institution: VANDERBILT UNIVERSITY NASHVILLE, TN
Project Period: 2000/04/01-2003/03/31

Multiple sclerosis (MS) is an inflammatory, demyelinating disease of the central nervous system (CNS). Although the etiology of MS is not known, there is considerable indirect evidence to suggest the role of infectious agents in the development of the disease. While a viral agent is still strongly considered, efforts to detect a virus in patients with MS have failed. We present preliminary data which argue that Chlamydiae Pneumoniae may be a candidate pathogen in MS. This inference is based on: a) the presence of PCR products to major outer membrane protein (MOMP) gene of C. pneumoniae in the cerebrospinal fluid (CSF) of patients with secondary progressive MS but not in other neurologic disease controls (OND); b) the presence of antibody to C. pneumonia antigens in the CSF of MS patients; and c) the presence of chlamydial antigens in brain autopsy specimens of patients who have died of MS. C. pneumoniae belongs to a genus of intracellular pathogens that are infectious to humans and other vertebrates. C. pneumoniae are implicated in many chronic diseases, including those presumed to be autoimmune. Chlamydiae cause chronic persistent inflammation in humans and other vertebrate animals and tissue injury in all cases appears to be immune mediated. Immune activation by C. pneumoniae includes induction of T cell response to heat shock proteins and production of pro-inflammatory cytokines. Our proposal sets out to meet the necessary criteria required to attribute a causal association between C. pneumoniae and MS. These would include the ability to detect the organism either directly by culture or by demonstrating the presence of C. pneumoniae DNA and mRNA and evidence of intracytoplasmic or organisms as proven by electron microscopy in the CNS tissue of patients with MS. Also, the presence of an immune response to C. pneumoniae that is contained within the CNS compartment will constitute strong circumstantial evidence of microbial infection.

Grant: 1R01NS038663-01A2
Program Director: NUNN, MICHAEL
Principal Investigator: MARRA, CHRISTINA M MD CLINICAL MEDICAL
SCIENCES, OTHER
Title: ROLE OF T PALLIDUM MSP-HOMOLOGUES IN CNS INVASION
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2000/09/22-2005/08/31

The goal of this application is to test two hypotheses: a) *T. pallidum* isolates contain populations of organisms with enhanced ability to invade the CNS; and b) The capacity for neuroinvasion is conferred by expression of specific surface proteins. Specifically, the Aims of this project are: 1) Compare the neuroinvasive capacity of *T. pallidum* subpopulations within strains using in vivo and in vitro models; 2) Establish highly neuroinvasive *T. pallidum* populations; 3) Demonstrate the direct involvement of surface proteins in mediating neuroinvasion in vivo; 4) Determine which tprs are most frequently transcribed and expressed by *T. pallidum* organisms that are neuroinvasive; 5) Identify proteins that are preferentially expressed by neuroinvasive organisms; 6) Investigate the direct involvement of the Tpr proteins and the proteins identified in Specific Aim 5 in neuroinvasion using in vitro and in vivo models. The studies proposed in this application may ultimately contribute to development of a vaccine for syphilis and may lead to the ability to identify patients at the highest risk for neuroinvasion. These investigations are most applicable to persons who are also infected with HIV-1, as they are at greatest risk for syphilis and neurosyphilis.

Grant: 2R01RR012294-04A1
Program Director: CARRINGTON, JILL L
Principal Investigator: RUBY, EDWARD G PHD OTHER AREAS
Title: VIBRIO VIRULENCE DETERMINANTS IN A BENIGN COLONIZATION
Institution: UNIVERSITY OF HAWAII AT MANOA HONOLULU, HI
Project Period: 1996/09/30-2005/02/28

DESCRIPTION: (Adapted from the Investigator's Abstract): The long term objectives of this research are to define the biochemical and genetic events that characterize the bacterial colonization of animal epithelial tissue. The benign, symbiotic infection of the light-emitting organ of the squid *Euprymna scolopes* by the luminous bacterium *Vibrio fischeri* will be used as a model system by which to address these objectives. Recent investigations of this association have centered on the events characterizing its initiation, establishment, and accommodation during the first 48 hours of the symbiotic infection of newly hatched juvenile squids. The experimental approaches described herein use bacterial mutants and host morphogenic indicators to manipulate and assay the complex succession of signalling and responses through which the host and bacterium communicate during these stages of the colonization process. The proposed research plan integrates 3 specific aims: (1) to determine how tissue tropism is facilitated by the host, and exploited by *V. fischeri* cells; (2) to investigate the role of host phagocytes in the association; and, (3) to define how the products of the *luxAIR* genes mediate both the swelling of crypt epithelial cells and the achievement of normal colonization levels by the bacteria. It is expected that the results of this research will provide insight into how newly discovered host-derived, mucus-like structures facilitate *V. fischeri* migration to internal host tissues, how the colonizing bacteria specifically evade the host immune system, and whether the oxygenase activity of their luciferase evokes both host tissue morphogenesis and nutrient provision. This system serves as a model of both benign and pathogenic *Vibrio* infection and, perhaps, of the evolution of the virulence state in vibrios. Specifically, the identification of bacterial determinants that potentiate light organ symbiosis has already revealed convergences with known *V. cholerae* virulence factors, and continued efforts may promote the discovery of as yet undescribed ones. The work proposed here will also provide new information about the mechanisms by which specific, stable colonizations of clams, snails, squid and their relatives may serve as environmental reservoirs for human pathogenic *Vibrio* species.

Grant: 1R21RR015104-01
Program Director: ROBINSON, JERRY
Principal Investigator: LEWINSOHN, DAVID M
Title: RHESUS MACAQUE MODEL OF TUBERCULOSIS
Institution: OREGON HEALTH & SCIENCE UNIVERSITY PORTLAND, OR
Project Period: 2000/09/01-2002/08/31

DESCRIPTION (Adapted from the Applicant's Abstract): Improved tuberculosis (TB) vaccines will be necessary to control the current pandemic of TB, which is out of control in much of the world. The development and pre-clinical evaluation of new TB vaccines require biologically relevant animal models in which to quantify the protective efficacy of candidate preparations. Much of the previous work in this area has been performed with two small animal models of TB, the mouse and the guinea pig. While these models have contributed much to our understanding of the determinants of vaccine-induced resistance to TB, neither may be a valid predictor of vaccine responses in humans. In addition, the co-existence of HIV and TB in many human populations may necessitate the evaluation of TB vaccines in a model of HIV infection. With regard to these latter two points, non-human primates have significant advantages over the small animal models currently employed. The long-term goal of this project is to develop a rhesus monkey model of TB and apply it to the evaluation of TB vaccine candidates. The two specific aims of the current proposal are: (1) to define the optimal dose of Mycobacterium tuberculosis delivered into the right lower lobe of the lung intratracheally which results in progressive pneumonia of at least three months duration; and (2) to define clinical endpoints (e.g., CT scans, quantitative culture of mycobacteria from BAL fluids and liver biopsies) which correlate with survival.

Grant: 1R03TW001235-01
Program Director: PRIMACK, ARON
Principal Investigator: CRAMER, WILLIAM A PHD
BIOPHYSICS:BIOPHYSICS-
UNSPEC
Title: SENSITIZED PHOTOINACTIVATION OF COLICIN E1 CHANNELS
Institution: PURDUE UNIVERSITY WEST LAFAYETTE WEST LAFAYETTE, IN
Project Period: 2000/02/01-2003/01/31

Some major problems of general interest from studies on the pore-forming colicins are: the nature of (i) the large soluble yields 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. 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. To initiate studies on the structure transition from closed- to open-channel state, 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. 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. Cross-linking of the polypeptide molecule (dimerization) was detected in parallel experiments with channel domain bound to liposomes. Based on studies of the Antonenko lab on the gramicidin cation-selective channel, it is proposed to investigate the mechanism of sensitized photoinactivation of the colicin E1 channel, and associated structure changes. The Trp-dependence of photoinactivation allows the use of single-Trp mutants to infer the helices and specific side chain involvement in channel formation. New approaches that will be developed in the course of this study will extend the application of Trp- and Cys-scanning mutagenesis. Preliminary experiments show also that colicin E1 membrane-binding and channel formation is affected by the lipid interfacial dipole potential. Both single- and multi-channel measurements will be used to investigate the mechanism photoinactivation and the role of the membrane dipole potential. Colicin photoinactivation will serve as an important model for study of photodamage of membrane proteins and photodynamic therapy, widely used in cancer treatment.

Grant: 1R03TW001244-01A1
Program Director: PRIMACK, ARON
Principal Investigator: HERNANDEZ, VICTOR J PHD
Title: HOST-DEPENDENT DETERMINANTS OF PHAGE LAMBDA DEVELOPMENT
Institution: STATE UNIVERSITY OF NEW YORK AT BUFFALO, NY
BUFFALO
Project Period: 2000/09/30-2003/08/31

The Escherichia coli bacteriophage lambda has played a crucial role in the development of molecular biology. Today it continues to serve as a paradigm for many diverse biological processes, including regulation of transcription and developmental biology. Several major early promoters are critical for phage (lambda) DNA replication and for the regulation of the "lysis-versus-lysogenization" developmental checkpoint. Our studies have revealed that activity of the lambda PR promoter, required for normal phage DNA replication, is severely inhibited in the presence of the RNA polymerase allosteric effector ppGpp, which accumulates in the host cell under conditions of nutritional stress. We have also shown that the activity of PR is dramatically stimulated by DnaA, the host replication initiator protein. It is interesting that DnaA binding, which leads to stimulation of PR, occurs atypically downstream of the transcription start point in the initial transcribed sequence. Furthermore, DnaA transcriptional activation of PR requires interaction with the RNA polymerase - subunit. The efficiency of establishing lysogeny, i.e., viral latency, following infection is found to be highly dependent on the host's intracellular levels of the stress signal effector, ppGpp. Apparently, the phage surveys ppGpp levels as an indicator of the host cell's physiological state. The activity of the three promoters, PE, PI, and PaQ, collectively determine the efficiency of lysogenization. We find that the activities of each of these promoters are differentially regulated by ppGpp. We propose here a systematic investigation of the regulation of transcription from lambda promoters: PR, PE, PI, and PaQ by ppGpp and/or DnaA. Both in vivo and vitro studies are planned. These will begin with genetic screening for mutant promoters with altered responsiveness to ppGpp and/or DnaA followed by detailed characterization by in vitro transcription assays. In addition, already isolated and B RNA polymerase mutants that mimic or bypass ppGpp-dependent functions will be used to characterize the physiological regulation of the lambda major early promoters both in vivo and in vitro. These investigations are aimed at discovering aspects of regulation of phage lambda development by ppGpp and DnaA, as model environmentally responsive and general gene regulators. How these host effectors modify the life cycle of the bacteriophage lambda and why, is a long-term objective of this proposal.

Grant: 1R03TW005575-01
Program Director: MICHELS, KATHLEEN M
Principal Investigator: RUSSELL, DAVID G PHD PARASITOLOGY, OTH
Title: Fatty Acid Catabolism & the glyoxylate shunt in Mycobact
Institution: CORNELL UNIVERSITY ITHACA ITHACA, NY
Project Period: 2000/09/01-2003/07/31

Description (adapted from the application): Isocitrate lyase (Icl) the first enzyme of the glyoxylate pathway is upregulated in *M. tuberculosis* when it is exposed to reduced oxygen tension or when exposed to an intracellular environment of activated macrophages. Icl competes with the Ich enzyme diverting the carbon from TCA cycle, which is otherwise wasted. The investigators suggest that Icl may utilize fatty acids found within the macrophages when they become the limiting carbon sources that explain their up-regulation and thus explain a probable mechanism by which *M. tuberculosis* can survive within hostile macrophages. The role of Icl in the pathogenesis of tuberculosis will be investigated under three specific aims of a funded RO1 grant addressing respectively the environmental inducers of Icl, role of Icl in maintaining latent infection and identifying inhibitors of Icl. This grant application will focus on identifying the substrates that feed the glyoxylate pathway using *M. smegmatis* and what this pathway does for the bacterium.