

FY01
NIH Extramural Support
in Bacteriology Research

Includes RPGs (Research Project Grants)
Excludes Clinical Trials

Grant: 2R01AG005731-12
Program Director: FULDNER, REBECCA A.
Principal Investigator: BONDADA, SUBBARAO PHD CELL BIOL
NEC:MOLECULAR BIOLOG
Title: Age Associated Changes in B Lymphocyte Function
Institution: UNIVERSITY OF KENTUCKY LEXINGTON, KY
Project Period: 1986/08/01-2006/07/31

DESCRIPTION (provided by applicant): Immune responses to pneumococcal polysaccharide antigens are important for protection against pneumococcal infections but are absent in neonates and are reduced in the aged. Using Pnu-Imune vaccine that is made up of 23 serotypes of pneumococcal polysaccharides and TNP-Ficoll, two type 2 thymus independent (TI)-2 antigens, we have shown that neonates and the aged have an accessory cell deficiency in addition to their B-cell defects. This deficiency is overcome by supplementation with accessory cell derived cytokines, IL-1 and IL-6. The neonatal macrophages make less IL-1 and IL-12 in response to TI stimuli such as TNP-Ficoll and lipopolysaccharide. This application is directed at testing the molecular basis of accessory cell defects leading to B-cell unresponsiveness to polysaccharide antigens in the neonates and the aged. It is hypothesized that toll-like receptor (TLR) expression and/or TLR signaling pathways may be defective in neonatal macrophages. Also, B-cell macrophage interactions required for TI responses may involve the macrophage derived cytokine BlyS and its B-cell receptors, TACI and BCMA, which may be dysfunctional in the neonate. Towards this goal five specific aims are proposed. 1. To determine if the defect in neonatal and the aged macrophages is limited to IL-1 and IL-12 or is a general property of macrophages in the neonate and the aged. 2. To test the hypothesis that the molecular nature of the defect in neonatal accessory cells that contributes to TI-2 unresponsiveness is in TLR expression or in the TLR signaling pathway. 3. To determine the role of dendritic cells in neonatal and aged unresponsiveness to polysaccharide antigens. 4. To determine if the restoration of neonatal B-cell responses to polysaccharides is due to their ability to signal neonatal B-cells/accessory cells via TLR signaling pathway. 5. To determine the role of monocyte-derived TNF like molecule, BlyS and its receptor (TACI), in B-cell-macrophage interactions and to determine if neonates or aged have defects in this ligand receptor system. Results from these studies should in future allow us to design and test agents that can augment accessory cell function and in turn to develop more effective polysaccharide vaccines for the neonate and the aged.

Grant: 1R01AG020239-01
Program Director: FULDNER, REBECCA A.
Principal Investigator: FERNANDES, GABRIEL
Title: Effect of Calorie Restriction on Infection During Aging
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR SAN SAN ANTONIO, TX
ANT
Project Period: 2001/09/30-2005/08/31

DESCRIPTION (provided by applicant): It is well established that calorie restriction (30-40 percent) prolongs the life span in rodents. Increased life span is accompanied by preventing the increase in body weight, maintaining cell-mediated immune function, and decreasing the incidence of malignancies and renal diseases. Although recent studies have revealed that CR alters the expression of various genes, particularly those involved in macromolecular damage, it remains unknown whether animals fed a lifelong CR diet are able to successfully ward off bacterial infection. Our recent studies showed that CR-fed young C57BL/6 mice are more susceptible to bacterial infection than AL-fed mice. The differences in susceptibility to infection could be due to differences in strains of mice, energy uptake, supplementation of vitamins and minerals or delayed maturity of humoral immunity. We, therefore, propose to compare 3 different commonly used diets for CR studies in 2 strains of mice (C57LBL/6 and Balb/C) which differ in their response to Th-1 and Th-2 cytokine expression. We will compare 1) the AIN-93 diet with and without additional vitamin supplements, 2) the AIN-93 CR diet with reduced carbohydrates but increased protein, fat and vitamins to equal the AL diet, and 3) NIH-3 1, an undefined but commonly used rodent chow diet for CR studies. We will measure the mortality rate from polymicrobial sepsis induced by cecal ligation and puncture (CLP) and from salmonellosis in young and old mice. To establish the susceptibility and resistance to infection both in young (8 mo) and old (24 mo) mice, we will carry out detailed functional studies of macrophages, Th-1 and Th-2 cytokine production, and cDNA superarray analysis for Th-1/Th-2 and inflammatory response cytokine genes. These studies will establish the role of CR in developing optimal immune function to ward off infection arising from common bacterial pathogens during aging. This new information may become very useful to prevent any sudden onset of infection during CR studies and/or studies of weight reduction either by diet or by drugs in humans.

Grant: 1R03AG018582-01
Program Director: NADON, NANCY L.
Principal Investigator: MORGAN, WILLIAM W PHD ANATOMY:ANATOMY
UNSPEC
Title: REGULATION OF GH BY A TETRACYCLINE-REPRESSIBLE SYSTEM
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR SAN SAN ANTONIO, TX
ANT
Project Period: 2001/02/01-2003/01/31

The purpose is to develop and initially characterize a novel transgenic mouse model in which the circulating levels of GH are normal but can be titrated downwards using a tetracycline-repressible transactivators system. Aim 1 is to develop a knockout-knockin transgenic mouse model which will [1] knockout the endogenous murine GH gene and [2] insert a DNA construct which will place the expression of the tetracycline (Tet)- repressible transactivator protein (tTA) under the control of the endogenous mouse GH promoter. The transactivator will in turn activate a minimal transactivator-dependent (TD) promoter to drive the expression of the rat GH gene (rGH). With this strategy the endogenous GH promoter will essentially drive the expression of rat GH. Since all of the normal regulatory mechanisms will be intact, GH levels should be approximately the normal levels found in the mouse and should show the pulsatile release characteristic of this hormone. Aim 2 will characterize and validate the model by first determining how well the knockout- knockin model recapitulates the normal secretion of GH. Plasma GH and insulin-like growth factor (IGF-1) will be measured in homozygous knockout-knockin versus wild-type mice. The rate of growth will also be assessed in these two groups of animals. Aim 2 will also determine if progressively higher dosages of Tet can be used to titrate the circulating levels of GH and IGF-1 in knockout-knockin mice. Initially, Tet will be administered in the drinking water. Should this approach prove to not be optimal, then subsequent studies will assess the ability of subcutaneously administered slow release tablets of Tet to titrate GH. The knockout- knockin mouse will be used in an ensuing R01 application to test the hypothesis that [1] reduced GH levels early in life may be advantageous to enhancing life span while [2] a moderate increase in GH levels in later life may also contribute to longevity.

Grant: 1R03AG019076-01
Program Director: FULDNER, REBECCA A.
Principal Investigator: JAGANNATH, CHINNASWAMY
Title: ROLE OF ADHESION MOLECULES IN REACTIVATION TUBERCULOSIS
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX
HOUSTON
Project Period: 2001/01/15-2002/12/31

Tuberculosis is the leading cause of death due to infectious and it has been estimated that 1/5th of global population is infected and at least 3 million people succumb to the disease. It is known to relapse among the elderly and the debilitated. Immuno senescence has been generally held to be responsible for enhanced susceptibility and investigators have used the mouse animal model to dissect the underlying mechanism. Previous studies using C57B1/6 mice have however yielded paradoxical results: both young and old C57B1/6 mice showed apparently normal macrophage function, T-cell receptor repertoire and application of memory responses following infection with MTB. However, unlike the younger generation, the older mice showed 'defects' in the expression of surface adhesions/intergrins on T-cells which normally enable homing and infiltration of T cells into lungs for effective control of lung tuberculosis. Dysregulation of adhesions/intergrins thus appears to play a pivotal role in immunosenescence and susceptibility, although the molecular mechanisms leading to the dysregulation remain enigmatic. We developed mouse models to illustrate acute, chronic and reactivation of tuberculosis using A/J and C57B1/6 mice. The A/J mice were found strictly more susceptible to tuberculosis and reactivated with TB faster compared to C57B1/6 mice. Increased susceptibility of A/J mice was associated with C5 deficiency as confirmed by using C5 knockout mice. Interestingly, C5 deficiency was also associated with a poor granuloma formation in the lungs following MTB infection and the disease occurred in mice, which were relatively young. These data together with the observation that C5 derived C5a can regulate the expression of adhesions/intergrins molecules suggested that immune responses are defective in A/J. Indeed, dysregulation of adhesions/intergrins in A/J may present as an accelerated aging phenomenon leading to enhanced susceptibility to intracellular pathogens. This investigation will therefore examine the hypothesis that C5 deficiency causes reduced expression of adhesions/intergrins molecules on T cells interfering with the structure and function of granuloma formation and ultimately with control of TB. Splenic lymphocytes from mice under varying stages of reactivation tuberculosis will be immunophenotyped using flow cytometry for distinct adhesions/intergrins molecules (e.g., ICAM-1, p selectin) that may suggest defects in infiltration and homing patterns.

Grant: 1R03AG019413-01
Program Director: MC CORMICK, ANNA M.
Principal Investigator: WARE, CAROL B PHD
Title: Inducible LIF Receptor Ablation in Adult Mice
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2001/07/01-2004/06/30

The ability to selectively, inducibly and reversibly target mutations to specific proteins in adult mice would be a powerful tool in the study of aging. Toward this goal, we have made a tetracycline responsive ablation of the gene for the leukemia inhibitory factor receptor (LIFR) in ES cells. This mutation has transmitted through the mouse germline and mating pairs heterozygous for the targeted mutation are now producing pups. Loss of LIFR using standard non-inducible gene targeting techniques is a perinatal lethal profoundly affecting many systems including bone (osteoporosis) and glial cell development (gliogenesis). Thus, we are now poised to address: 1. the utility of a tetracycline inducible gene ablation approach in the study of aging 2. identification of adult consequences of LIFR loss. The inducible targeting vector incorporates a complete set of tet-off elements so that a full length rat LIFR cDNA is incorporated homologously into exon 2 of the mouse LIFR effectively ablating the mouse gene with tetracycline control of the introduced rat LIFR homolog. Because rat LIFR insertion is targeted to be under appropriate control of the endogenous mouse LIFR promoter elements, expression of rat LIFR is on where murine LIFR is constitutively expressed in the absence of a tetracycline derivative, doxycycline (Dox) and silenced in the presence of Dox. Expression of rat LIFR is reactivated upon removal of Dox. Sensitivity of this system will be studied both by semi-quantitative reverse transcription polymerase chain reaction (rtPCR) to measure whole tissue alterations in LIFR levels in response to Dox in the drinking water and by utilizing a beta-galactosidase reporter gene incorporated in the targeting construct which is also switched off in the presence of Dox. Beta-galactosidase will be visualized in situ by X-gal staining to analyze localized effects of Dox administration. Effects of Dox and preliminary analysis of biological consequences of adult LIFR ablation will be assessed in bone, the central nervous system, skeletal and cardiac muscle, lung, liver, pancreas, spleen and kidney. In summary, a new technique for adult genetic manipulation will be developed and characterized that will elucidate the role in aging of the multi-functional cytokines that utilize LIFR.

Grant: 1R15AG018320-01A1
Program Director: FULDNER, REBECCA A.
Principal Investigator: FRESA, KERIN L PHD
Title: Immunosenescence and Chlamydia Pneumoniae
Institution: PHILADELPHIA COLLEGE OF OSTEOPATHIC MED PHILADELPHIA, PA
Project Period: 2001/05/01-2004/04/30

DESCRIPTION (the Applicant's Abstract): With increasing age, there is increased incidence and severity of infectious diseases, including pneumonia, meningitis and sepsis, as well as many non-infectious diseases including cancers, heart disease, Alzheimer's disease and other dementias. The obligate intracellular bacterial parasite Chlamydia pneumoniae is an established pathogen for respiratory infection. Recent evidence from a number of laboratories suggests that C. pneumoniae may be a factor in the pathogenesis of a number of non-respiratory diseases including Alzheimer's disease and atherosclerosis. It has been repeatedly hypothesized that increased incidence of infectious and other diseases with age may be the result of age alterations in the immune system, particularly in cell mediated immune reactions. It is well established that T cell function, as measured in vivo by delayed type hypersensitivity reactions and in vitro as proliferative responses to antigenic or mitogenic stimulation, both decline with age. While infection with C. pneumoniae as well as other Chlamydial species induces production of antibodies, recent evidence suggests that cell mediated immune mechanisms play a key role in recovery from infection as well as immunopathology associated with Chlamydial infection. It has not been established whether old animals are able to clear infection by C. pneumoniae or remain chronically infected. It also remains unknown whether the immune response, particularly the cell-mediated immune response, to acute chlamydial infection is altered with age. Finally, the extent to which the cell-mediated immune response to Chlamydia, or lack thereof, contributes to the pathogenesis of diseases such as atherosclerosis or Alzheimer's disease is entirely speculative. We propose here to begin to address these issues in a mouse model. The working hypothesis is that the cellular immune response against C. pneumoniae declines with age. The Specific Aims of this project are: 1) To assess whether aging is associated with alterations in the clinical course of experimental intranasal infection of mice by C. pneumoniae; and 2) To assess whether there are age-associated changes in the immune response to C. pneumoniae. Specifically, we will examine proliferative responses, generation of cytotoxic T cells, and production of Th1 associated cytokines (IL-2 and IFN-g).

Grant: 1P01AI046422-01A1
Program Director: KLEIN, DAVID L
Principal Investigator: MURPHY, TIMOTHY F
Title: Recurrent otitis media and COPD: immunity and vaccines
Institution: STATE UNIVERSITY OF NEW YORK AT AMHERST, NY
BUFFALO
Project Period: 2001/08/15-2005/07/31

Otitis media leads to enormous morbidity and to direct annual healthcare costs estimated to be \$3 billion in the U.S. Chronic obstructive pulmonary disease (COPD) is the fourth most common cause of death in the U.S. Bacterial infection in COPD causes substantial morbidity and mortality. Two of the three most common bacterial pathogens to cause otitis media and respiratory tract infections in COPD are non-typeable *Haemophilus influenzae* (NTHI) and *Moraxella catarrhalis*. The development of vaccines to prevent otitis media and respiratory tract infections in adults with COPD would have important impact in reducing mortality, preventing morbidity and reducing healthcare costs in these settings. This program project proposes studies which will elucidate the immune response to specific antigens of NTHI and *M. catarrhalis*. Our hypothesis is that the pattern of respiratory tract infections due to NTHI and *M. catarrhalis* in children and adults depends on the immune response to specific surface antigens of the organisms. The aims of the project will be accomplished through the efforts of a multi-disciplinary research team which will collaborate to carry out three interrelated projects. Project 1 will characterize the role of human T cell responses to protein P6, a promising vaccine antigen will be determined through studies with carefully defined samples from humans. Project 3 will focus on antigenic characterization of the lipooligosaccharide (LOS) of *M. catarrhalis*, the role of LOS as an adhesin and the human immune response to determinants on the LOS molecule. An Administrative/Statistical Core will coordinate the program and provide statistical expertise to each of the projects. Since NTHI and *M. catarrhalis* are exclusively human pathogens, a strong emphasis is placed on elucidation of the human immune response. The proposed studies will advance the field of vaccine development to prevent otitis media and respiratory tract infections in COPD.

Grant: 2R01AI007194-36

Program Director: KORPELA, JUKKA K.

Principal Investigator: HELINSKI, DONALD R PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC

Title: Structure and Genetic Control of Colicines

Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA

Project Period: 1978/02/01-2006/04/30

The research in this proposal is concerned with genetic and biochemical mechanisms responsible for the initiation of replication and partitioning of the broad-host-range plasmid RK2 in *Escherichia coli* and distantly related Gram-negative bacteria. Plasmid RK2 specifies resistance to the antibiotics ampicillin, tetracycline and kanamycin, and will replicate and is stably maintained in a wide range of Gram-negative bacteria. Mechanisms of plasmid replication initiation and segregation to daughter cells will be investigated in *E. coli*, *Pseudomonas putida* and *Pseudomonas aeruginosa* using biochemical, genetic and cytological techniques. RK2 encodes a replication initiation protein (TrfA) and a replication origin that has as its main features 17 base pair repeats (iterons) that are bound by the TrfA protein, four DnaA boxes, and an A+T rich sequence that contains four 13-mer sequences. The plasmid also contains two regions, including the *par* operons, which are involved in stable maintenance. A major thrust of the proposed research is understanding the unique properties of this plasmid that account for its ability to initiate its replication and faithfully partition itself during cell division in a wide range of bacteria. To this end, the activities of the key host proteins DnaA and DnaB of *E. coli*, *P. aeruginosa*, and *P. putida* along with the plasmid specific initiation protein in the initiation of replication of RK2 and narrow-host-range plasmids P1 and F will be determined. In addition, the activities of these various host proteins (along with the DnaC protein of *E. coli*) at the chromosomal replication origins of *E. coli* and the two *Pseudomonas* strains will be compared. Both FISH and GFP-tagging techniques will be used to localize the RK2 plasmid in wild-type and mutant *E. coli* strains and in bacteria distantly related to *E. coli*. GFP-tagging of RK2 and of specific replication proteins will be used for time-lapse analysis of the dynamic movement of this plasmid and replication proteins during cell growth and division. These various studies should contribute to our understanding of the fundamental processes of initiation of DNA replication, DNA segregation, and the dissemination of antibiotic resistance in bacteria.

Grant: 2R01AI009644-32
Program Director: KORPELA, JUKKA K.
Principal Investigator: NIKAIDO, HIROSHI MD
MULTIDISCIPLINARY: MULTIDISCIPLINARY
LIN, BASIC MED
Title: BIOCHEMISTRY OF BACTERIAL CELL MEMBRANES
Institution: UNIVERSITY OF CALIFORNIA BERKELEY BERKELEY, CA
Project Period: 1976/03/01-2006/02/28

The overall goal of this project is to study the flux (both in and out) of small molecules across the surface layers of bacteria. As is well-known, multiple resistance in pathogenic microorganisms is becoming a serious threat to human health. Recent studies in our own and other laboratories suggest that in many cases such resistance is the synergistic effect of two factors, the cell surface layer acting as permeability barrier for the influx of drugs (outer membrane of Gram-negative bacteria or mycolate-containing cell wall of mycobacteria) and active, multidrug efflux pumps. In fact, for more advanced antimicrobial agents that resist the enzymatic inactivation of bacteria, such as fluoroquinolones, this mechanism that prevents the access of drugs to the target has become a primary mechanism of resistance. In this study, we plan to continue our characterization of both of these resistance mechanisms. Thus we will characterize the "slow" porins of organism such as *Pseudomonas aeruginosa*, the proteins that contribute to the generalized intrinsic resistance of these bacteria by slowing down the influx of antimicrobial agents across their outer membrane. We will study the mechanism in which the cell wall of mycobacteria drastically slows down the entry of most agents by producing an organized layer of mycolic acid and other lipids. At the other end, we will investigate the mechanism of multidrug efflux pumps, especially those pumps that show an incredibly wide range of specificity, such as AcrAB of *Escherichia coli* that extrudes almost any lipophilic or amphiphilic compounds, including dyes, disinfectants, detergents, solvents, and practically all antibiotic (except aminoglycosides). These pumps appear to become expressed more strongly when the bacteria are under stress, and the pathway of regulation will be defined. Finally, as a prototype of ABC transporter, which includes P-glycoprotein and CFTR, the maltose transporter complex of a hyperthermophile *Pyrococcus furiosus* will be purified, because proteins from hyperthermophiles tend to produce well-diffracting crystals, and there is great need to obtain crystallographic data on this important class of transporters.

Grant: 2R01AI016963-19A1
Program Director: WALI, TONU M.
Principal Investigator: MURRAY, HENRY W MD MEDICINE
Title: HOST IMMUNOREGULATION OF ANTILEISHMANIAL CHEMOTHERAPY
Institution: WEILL MEDICAL COLLEGE OF CORNELL NEW YORK, NY
UNIV
Project Period: 1980/09/30-2006/03/31

DESCRIPTION (provided by the applicant): Since there is no vaccine to prevent visceral leishmaniasis (VL) (kala-azar), a disseminated intracellular protozoal infection, its practical management revolves around successful drug therapy. Pentavalent antimony (Sb) remains the conventional, albeit suboptimal and now less effective, treatment of choice. To formulate new therapeutic strategies in kala-azar, the two logical complementary approaches are (a) new drug development (amphotericin B (AmB), miltefosine) and (b) identification of immunologic mechanisms which can be regulated and translated into treatment. This application focuses on immuno-regulation of successful host responses to antileishmanial chemotherapy and is directed at the effects of T cells and cytokines. This area of research, immunochemotherapy, is relevant to the field with future as well as already-realized clinical impact. The overall objective is to optimize the host response to treatment by characterizing and then manipulating key immuno-regulatory mechanisms in favor of the host receiving chemotherapy. The unifying hypothesis is that, once targeted, discrete mechanisms can be stimulated or inhibited and then joined with chemotherapy to enhance initial intracellular *Leishmania donovani* killing in the tissues and also prevent post-treatment relapse. To accomplish the objective and test this translational research strategy, this work will be carried out in a clinically relevant sequential fashion and will (a) be directed at in vivo responses, (b) test hypotheses in established infection and (c) incorporate more than one chemotherapeutic agent to probe the host response to current (Sb) as well as more newly used treatments (AmB). Three related Specific Aims support the overall objective and will advance the analysis: Aim 1: Determine how IFN-gamma (Th1 cell-associated response) regulates the response to Sb and converts its action from leishmanistatic to leishmanicidal. Aim 2: Test the hypothesis that synergy with antileishmanial chemotherapy can be induced by specific, linked pharmacologic intervention designed to raise intrinsic host T cell reactivity by: (a) neutralizing the suppressive effect of endogenous interleukin 10, (b) enhancing interleukin 12 action by inhibiting cyclooxygenase-2 (COX2), and (c) triggering antileishmanial T cell costimulatory pathways, CD28:B7 and CD40L:CD40. And Aim 3: Characterize immunostimulation in AmB's efficacy, and in recrudescence infection following AmB therapy, pinpoint the likely cytokine-driven T cell mechanism which prevents post-treatment relapse.

Grant: 2R01AI018000-20

Program Director: KLEIN, DAVID L

Principal Investigator: HEWLETT, ERIK L MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC

Title: BORDETELLA CYCLASE-STRUCTURE AND BIOLOGICAL ACTIVITIES

Institution: UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA
CHARLOTTESVILLE

Project Period: 1980/09/01-2005/12/31

DESCRIPTION (Adapted from the Applicant's Abstract): Adenylate cyclase toxin (ACT) is a fascinating bacterial virulence factor produced by *Bordetella pertussis*. It has unusual features, which make it an important prototype for study of the actions of several families of toxins. ACT is a 1706 amino acid protein that possesses an adenylate cyclase domain responsible for catalyzing the production of cAMP after it is delivered to the cytoplasm of the target cell. In addition, this molecule is a member of the RTX family of bacterial toxins and proteins, characterized by their calcium-binding repeats. Many of the family members are pore-forming hemolysins and cytotoxins and ACT is also hemolytic, apparently by formation of an oligomeric pore in the host cell membrane. In addition, this toxin elicits K⁺ efflux from host cells in a process that precedes and can be dissociated from oligomer formation. Work over the last several years has led to recognition that these activities of ACT are separate and distinct. In response, the objective of the next phase of this ongoing project is to understand the individual steps of toxin action and how they relate to one another. To accomplish this goal, the research will follow these Specific Aims: 1) physical characterization of ACT as it is prepared and evaluation of whether any or all of the activities are dependent on the formation of dimers by the toxin; 2) evaluation of the initial interaction of ACT with the target cell, using methods to define the early step before any membrane insertion is able to occur; 3) mapping to learn which portion of the toxin is inserted into the membrane, using electron paramagnetic resonance (EPR) spectroscopy, and determination of the relationship of that event to K⁺ efflux; 4) mapping of the portion of the catalytic domain which is delivered to the cytoplasm and investigation of whether intoxication and K⁺ efflux/hemolysis are mutually exclusive events for any single toxin molecule; and 5) characterization of the process of oligomerization and the domains of the toxin involved. This work is directed at understanding the mechanism of action of this toxin at a very basic level, with novel approaches and technologies. The results will be important for defining the role of this toxin in the pathogenesis of pertussis and for gaining better knowledge of its actions as a novel biomedical research probe and vehicle for delivery of foreign antigens to the host immune system. In addition, the information acquired from the proposed studies will contribute to the general field of toxin research, in which there are many examples of large extrinsic proteins gaining access to host cells by incompletely understood mechanisms.

Grant: 2R01AI019716-19
Program Director: SAWYER, LEIGH A.
Principal Investigator: KAPER, JAMES B PHD
MICROBIOLOGY:BACTERIOLOGY
Title: DEVELOPMENT OF A LIVE ORAL CHOLERA VACCINE
Institution: UNIVERSITY OF MARYLAND BALT PROF SCHOOL BALTIMORE, MD
Project Period: 1983/01/01-2006/01/31

DESCRIPTION (Adapted from the Applicant's Abstract): An ideal vaccine for the prevention of cholera is not yet available. Previous work in this project has resulted in the development of an attenuated live oral cholera vaccine, V. cholerae CVD 103-HgR. This vaccine confers strong protective immunity against experimental challenge with virulent V. cholerae O1 after a single dose. Although this vaccine is highly protective in North American volunteers and has been licensed in several highly developed countries for protection of travelers to cholera endemic countries, a recent field trial of this vaccine in Indonesia failed to show efficacy. The development of attenuated cholera vaccines has been plagued by the fact that V. cholerae strains deleted of the ctx genes encoding cholera toxin can still produce varying amounts of diarrhea and non-diarrheal symptoms such as headache, fever, abdominal cramps, and malaise in many individuals. Such symptoms are not seen with CVD 103-HgR, in all probability because this strain colonizes the human intestine at greatly reduced levels compared to the reactogenic, avidly colonizing ctx-negative strains. Although the reduced colonization of CVD 103-HgR was still sufficient to engender a protective immune response in North American volunteers whose small bowel intestinal flora is relatively sparse, it was not sufficient to induce a protective immune response in a cholera-endemic population with a heavy burden of small bowel intestinal flora which would compete against a live oral vaccine strain. The ability to construct a better-colonizing strain is hampered by the uncertainty as to what bacterial factor is responsible for the reactogenicity. Thus, the next period of support for this project will focus on characterizing the response of epithelial cells to adherent V. cholerae, establishing the role in reactogenicity of various cytotoxins, proteases, other degradative enzymes, and other potential toxins revealed by the recently completed genome sequence of V. cholerae, and determining the V. cholerae genes that are specifically expressed during the course of human infection. These studies will use the broadest possible range of models to study host-pathogen interactions, including intestinal epithelial cell lines, freshly harvested human intestinal tissue, animal models, and human volunteer studies.

Grant: 2R01AI022383-14A2

Program Director: MILLER, MARISSA A.

Principal Investigator: SALYERS, ABIGAIL A PHD
MICROBIOLOGY:MICROBL
BIOCHEMISTRY

Title: Conjugal Transfer of Bacteroides Antibiotic Resistances

Institution: UNIVERSITY OF ILLINOIS URBANA- CHAMPAIGN, IL
CHAMPAIGN

Project Period: 1985/09/30-2006/05/31

DESCRIPTION (provided by applicant): The incidence of antibiotic resistance is increasing in many groups of disease-causing bacteria. Although some bacteria become resistant to antibiotics through mutation, acquisition of antibiotic resistance genes from other bacteria is probably more common. This proposal focuses on the genus *Bacteroides*. *Bacteroides* species are among the numerically predominant species of bacteria in the human colon. *Bacteroides* species are also opportunistic pathogens that can cause life-threatening infections. Many *Bacteroides* strains have become resistant to multiple antibiotics. Transfer of resistance genes among these strains appears to have occurred mainly through the actions of a group of conjugative transposons (CTns), represented by CTnDOT. CTnDOT excises from the chromosome to form a circular intermediate, which transfers by conjugation to a recipient and integrates into the recipient's chromosome. During the previous funding period, genes responsible for excision and transfer were identified and shown to be controlled by a complex set of regulatory genes. These genes may allow the CTn to coordinate excision and transfer so that nicking to initiate transfer of the circular form does not occur until excision is complete. Both excision and transfer are stimulated over 1,000-fold by the antibiotic tetracycline. Previously, we found that three genes, *rteA*, *rteB* and *rteC* function as central regulatory genes. We propose that *RteC* triggers expression of two excision genes, *excA* and *excB*. The first specific aim of the proposal is to test this hypothesis. The second aim is to determine how *ExcA* and *ExcB*, presumably in concert with the CTn integrase (*Int*), catalyze excision and circularization of the CTn. The third specific aim is to determine whether *RteC* also controls expression of a gene currently designated as *orf5* and to test the hypothesis that *Orf5* in turn controls the expression of transfer (*tra*) genes. The fourth specific aim is to answer the question of how effective coordination of excision and transfer actually is. The last aim is to define the characteristics and functions of *RteA* and *RteB*, which control expression of *rteC*.

Grant: 2R01AI023545-16
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: BLOOM, BARRY R PHD
MICROBIOLOGY:IMMUNOLOGY
Title: Mycobacterial Genes, Antigens, and Vaccines
Institution: HARVARD UNIVERSITY (SCH OF PUBLIC HLTH) BOSTON, MA
Project Period: 1986/04/01-2006/05/31

DESCRIPTION (provided by the applicant): Tuberculosis and HIV/AIDS represent the major infectious causes of death in the world, and tuberculosis (TB) is the attributable cause of death in a third of AIDS patients in Africa. Drug resistance to TB is emerging in Europe and Asia, drug treatment regimens are long and expensive and compliance is limited. For these reasons, we propose to bring a multidisciplinary approach, joining molecular genetics and immunology, to developing safe and effective live attenuated vaccines against TB. Since peak age of disease is 15-25y, we believe a live attenuated vaccine that induces long enduring immunological memory will provide the most useful protection against disease. In previous work we have developed tools to genetically manipulate slow growing mycobacteria, including the capability of creating specific deletion mutants to attenuate virulent M tuberculosis. One aim is to test the hypothesis that M tuberculosis represents a better vaccine candidate than BCG, to create and test auxotrophic mutants, growth mutants and persistence mutants of M. tuberculosis for safety and immunogenicity in mice. A second is to determine the optimal duration of growth of vaccine strains in vivo for the development of immunological memory responses, particularly through the use of regulated promoters. This will also allow us to elucidate similar requirements for producing tissue damage. Our final aim remains to understand the immunological mechanisms of protection against experimental tuberculosis, particularly exploring the role of innate responses mediated by the Toll-like receptor family, the minimum epitope and antigen requirements for protection, and the possible role of cytotoxic T-lymphocytes (CTL) in protection.

Grant: 2R01AI023695-15
Program Director: KLEIN, DAVID L
Principal Investigator: WEISS, ALISON A
Title: Biogenesis of Pertussis Toxin
Institution: UNIVERSITY OF CINCINNATI CINCINNATI, OH
Project Period: 1986/07/01-2006/05/31

DESCRIPTION (provided by the applicant): Pertussis toxin is the major virulence factor of *Bordetella pertussis*. It is the most complex toxin known. Five different subunits associate in an unusual, 1:1:1:2:1 ratio. The subunits are secreted to the periplasm by the Sec-pathway where intramolecular disulfide bonds form and the subunits assemble into the functional toxin. Nine Ptl (for pertussis toxin liberation) proteins mediate secretion of properly assembled toxin past the outer membrane. Specific Aim 1. Characterization of periplasmic folding and assembly of pertussis toxin. We have shown that disulfide bond formation occurs by an unusual pathway in *Bordetella pertussis*. The role of the novel periplasmic folding chaperones, and small thiols in the assembly of pertussis toxin will be investigated. Specific Aim 2. The role of individual Ptl proteins in secretion. The function of the nine Ptl proteins will be investigated using genetic and biochemical methods with the goals of identifying the channel forming proteins, the glycohydrolase, proteins involved in assembly of the secretion complex, and proteins involved in specificity (e.g. distinguishing individual subunits from assembled pertussis toxin). Specific Aim 3. Horizontal transfer of pertussis toxin genes. Two non-functional pertussis toxin operons (the chromosomal operon in *Bordetella bronchiseptica*, and the phage-encoded operon in *Bordetella avium*) will be investigated to gain an understanding of the unique events that occurred in the species *B. pertussis* that permit only them to express pertussis toxin. It is our hope that a detailed molecular understanding of the pertussis toxin secretion process will lead to better treatments for toxigenic diseases, including new therapeutics to block secretion, or "anti-virulence" therapies that could be as effective as the current anti-microbial therapies.

Grant: 2R01AI024870-14
Program Director: LANG, DENNIS R
Principal Investigator: SCOTT, JUNE R PHD
MICROBIOLOGY:BACTERIOLOGY
Title: REGULATION OF EXPRESSION OF ADHERENCE FACTORS
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 1988/02/01-2006/01/31

DESCRIPTION (Adapted from the Applicant's Abstract): Enterotoxigenic *Escherichia coli* (ETEC) are a major cause of diarrheal disease in infants and small children and in travelers to developing countries, including military personnel. The first step in establishment of infection is attachment of the pathogen to the host tissue, which is believed to be mediated by pili (and/or thinner, more flexible fibrillar structures). The major long-term goals of this work are to understand the mechanisms by which pili are synthesized and the mechanisms that regulate their synthesis. A limited number of serologically different pili prevalent among ETEC strains isolated from human disease include a group encoded by homologous genes, for which CS1 serves as the prototype. The investigators found that the genes encoding CS1 are positively regulated by Rns, which shows homology to AraC. The investigators have found that autoactivation by Rns involves DNA regions both upstream and downstream of the regulated promoter. Although this is unprecedented, the investigators found that many Rns-related global regulators of virulence determinants of enteric pathogens can substitute for Rns and therefore act similarly. The investigators wish to study regulation of expression of Rns and of CS1 pili further. Aim I addresses the regulation of Rns. In other systems activated by a Rns-related protein, more than one virulence operon is usually controlled. Preliminary work indicates that this appears to be the case in ETEC as well. Aim II proposes to continue this work with the hope of identifying other virulence factors of ETEC and better understanding the pathology of the infections ETEC cause. The investigators have found that the genes needed for synthesis of human ETEC pili are completely unrelated to those of other pili and CS1 pili appear to be much simpler structures, so it seems possible that a detailed understanding of their morphogenesis is within reach. Aims III and IV of this proposal are directed at approaching this. The investigators hope that this work will suggest new approaches to development of human anti-ETEC vaccines and therapies, as well as providing a greater understanding of protein interaction and assembly of multi-protein structures in bacteria.

Grant: 2R01AI028927-11A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: HATFULL, GRAHAM F
Title: Molecular Biology of Mycobacteria and Their Phages
Institution: UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PA
PITTSBURGH
Project Period: 1989/12/01-2005/06/30

DESCRIPTION (provided by the applicant): Mycobacterium tuberculosis, the causative agent of human tuberculosis, kills more people than any other single infectious agent. The prevalence of TB is greatest in the developing world but its control in the United States has become severely complicated by the appearance of multidrug resistant strains of M. tuberculosis. There has recently been a sharp increase in the incidence of these MDRTB strains in developing countries. Effective control of tuberculosis requires improved and more rapid diagnostic methods, more efficacious vaccines, and better antimycobacterial drugs, particularly for treatment of multidrug resistant infections. With the recent advances in mycobacterial genetics and the determination of the complete genome sequence of M. tuberculosis, there is now renewed hope that a more sophisticated understanding of the physiology, genetics, and metabolism of M. tuberculosis will lead to novel strategies for controlling mycobacterial infections. Unfortunately, in spite of these genetic tools and genomic information, we know little about the molecular basis of the fundamental aspects of mycobacterial physiology-such as slow growth, their unique cell wall, and DNA replication-let alone the molecular basis of mycobacterial pathogenesis. Viruses are powerful tools for genetic analysis of a broad range of organisms, and the viruses of mycobacteria (mycobacteriophages) are no exception. The use of mycobacteriophages was instrumental in the establishment of mycobacterial genetics and the creation of cloning vectors for the introduction of DNA into mycobacteria. More recently, recombinant reporter mycobacteriophages have been proposed as clinical tools for rapid determination of drug susceptibilities of clinical isolates of M. tuberculosis. This project aims at understanding the intimate interface between mycobacteriophages and their hosts. This interaction begins with the association of free phage particles with bacterial cells followed by injection of phage DNA into the cell. Phage DNA may then either integrate into the host genome and be genetically silenced, or reprogram the cell to direct it towards phage gene expression and subsequent cell lysis. By exploring these events we will gain insights into the regulation of gene expression, the structure of the mycobacterial envelope, and the process of phage-mediated cell lysis. We will also use proteomic approaches to understand the influence of phage gene expression on that of its host.

Grant: 2R01AI029040-10A1
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: 1989/12/01-2006/03/31

DESCRIPTION (Verbatim 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 ca. 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 specific 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 "nontypeable" strains carry the genes for CP5 or CP8 production, we will determine why capsule is not expressed. We also plan to evaluate nontypeable 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: 2R01AI029611-11A1
Program Director: KLEIN, DAVID L
Principal Investigator: STULL, TERRENCE L
Title: H.influenzae Hemoglobin/Hemoglobin-Haptoglobin Binding
Institution: UNIVERSITY OF OKLAHOMA HLTH SCIENCES CTR OKLAHOMA CITY, OK
Project Period: 1990/12/01-2006/05/31

Current vaccines against *Haemophilus influenzae* type b reduced the incidence of *H. influenzae* invasive diseases. However, nontypeable *H. influenzae* are a common cause of pneumonia and otitis media, which is associated with hearing loss and language deficits. *H. influenzae* has an absolute growth requirement for heme and the human body is its sole niche. Previously, we characterized a family of genes encoding proteins, Hgp, which bind human hemoglobin and the hemoglobin-haptoglobin complex. Mutation of all the hgp genes in a strain does not abrogate hemoglobin binding. We have identified a putative gene product encoding this residual hemoglobin utilization activity. Expression of Hgps is repressible by heme, but not by elemental iron. The upstream region of each gene lacks the Fur consensus site suggesting that the Fur repressor does not directly regulate the Hgps. We have isolated *H. influenzae* fur mutants and fur-independent mutants with altered hgp expression. Thus, regulation is more complex than the classic ferric uptake repressor system described for a wide range of bacterial species. Sequence analysis of Hgps reveals four highly conserved regions. Preliminary data about the complex regulation of hgp expression, localization of a binding region of HgpA, and the identification of conserved regions provide an opportunity to investigate the structure/function, gene regulation, and immunobiology of the Hgps. The current project will determine ligands bound by Hgp, identify binding sites, characterize the conserved regions, and determine the relative growth advantage of multiple Hgps in a strain. In addition, the role of fur and fur-independent elements in the regulation of Hgp will be examined. Finally characterization of the immunobiology of the Hgps will determine the protective capacity of antisera to the conserved regions of the Hgps in animal models of invasive and noninvasive disease. These experiments will provide insight into how heme acquisition is related to pathogenicity. These studies will lay the foundation for long-term studies focusing on prevention of all *H. influenzae* related disease.

Grant: 2R01AI029733-11A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: LOVETT, MICHAEL A MD INTERNAL
MED:INFECTIOUS DISEASE
Title: Pathogenic Mechanisms in Lyme Borreliosis
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 1990/06/01-2006/03/31

DESCRIPTION (provided by the applicant): This proposal is centered on the molecular basis of host-adaptation by *Borrelia burgdorferi* in mice and on the phenotypic changes of host-adapted *Borrelia* (HAB) that relate to pathogenesis and immunity. The small numbers of *B. burgdorferi* (Bb) present in infected mice has been a hindrance to determination of their surface antigenic structure. The signals in the host environment that initiate the process of host-adaptation by Bb have not been reported. Several recent findings in our laboratory form the basis for this proposal. We have learned that the magnitude of spirochetemia in scid mice is orders of magnitude greater than previously appreciated, making possible direct study of HAB spirochetes. These HAB circulating in the blood of scid mice are bound to blood cells, and have an antigenic composition distinct from that of in vitro cultivated Bb. Further, we learned that contact of in vitro cultivated Bb with blood cells results in expression of proteins otherwise poorly expressed in vitro, but upregulated during infection. Three specific aims are proposed. The first is "proteomic analysis of Bb host-adaptation in the mouse model of Lyme disease." We have developed novel methods for efficient extraction of the surface proteins expressed by HAB in mouse skin, blood, heart, and joint. Using the tools of proteomics we will catalogue the full set of HAB surface proteins expressed in different tissues and the relative amounts in which they are expressed. The second specific aim is "molecular basis of host-adaptation." Contact with host cells in vitro triggers Bb to upregulate the expression of certain surface antigens. These events will be related to the findings of our proteomic analysis of host adaptation in the mouse. The Bb receptor(s) and host cell ligand(s) that mediate this process will be defined. The third specific aim is "relationship of surface antigenic structure of host-adapted Bb to protective immunity." HAB bound to circulating blood cells will be used to assess the relative representation of specific molecules on their surface. Novel HAB surface proteins will be tested as protective immunogens.

Grant: 2R01AI030138-11A1

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: 1991/09/01-2006/02/28

DESCRIPTION (provided by the applicant): This project is a continuation of a broadly based investigation into the regulatory mechanisms that control the production of virulence factors and other exoproteins in *Staphylococcus aureus*, with an extension to the regulation of superantigen toxin synthesis in *Streptococcus pyogenes*. The present focus is on the mechanism of action of the agr effector molecule, the 514 nt RNAIII, and is based on the hypothesis that RNAIII interacts with internal regulatory mediators. We have found that a key intracellular mediator is the 2-component sae system, which is transcriptionally activated by RNAIII and which is required for the expression of many of the exoprotein genes. There are four specific aims: 1. To characterize the central pathway from agr via RNAIII to target genes. 2. To determine the mechanisms by which external stimuli and certain growth conditions affect the expression of genes in the agr regulon. 3. To analyze by means of reporter gene fusions the expression of different regulatory and target genes. 4. To determine whether the regulation of speA and other toxin genes by group A hemolytic streptococci is similar to staphylococcal regulation of virulence factors. In Aim 1, oligonucleotide arrays will be used to define the agr regulon and overlapping regulons governed by other global regulators of virulence. In Aim 2, the mechanism by which RNAIII activates the intracellular mediator sae will be identified, and its role in regulating target gene transcription defined. In Aim 3, the focus will be on the mechanism by which certain external stimuli interact with the agr pathway, starting with a determination of the sets of genes (stimulons) that are affected by particular environmental conditions. It is proposed to compare the effects of these stimuli and of various regulatory mutations in planktonic cultures with their effects in biofilms. In Aim 4, a continuation of a study in which we have identified an autoinducer of streptococcal erythrogenic toxin (SPEA) synthesis, is proposed.

Grant: 2R01AI030479-12
Program Director: LANG, DENNIS R
Principal Investigator: MILLER, SAMUEL I
Title: PHOP REGULON AND SALMONELLA VIRULENCE
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 1991/02/01-2006/05/31

DESCRIPTION (provided by the applicant): Salmonella are facultative intracellular pathogens which cause significant diseases in humans and animals. These organisms are responsible for several disease syndromes, including enteric (typhoid) fever, gastroenteritis, bacteremias and focal infections. Typhoid fever is a severe systemic illness which is mostly a problem in the developing world and in travelers. Non-typhoidal salmonella infections are increasing in the USA and are largely associated with contaminated food. Salmonellae infections are most severe in infants, the elderly, and in immunosuppressed individuals. This grant proposes to study the mechanism by which Salmonellae survive host innate immune killing. Innate immune killing involves the non-antigen specific mechanisms by which animals eliminate invading bacteria. Included in innate immune mechanisms are antimicrobial peptides produced at mucosal surfaces and within phagocytic cell granules and cytokines produced in response to recognition of bacterial lipid A. Pathogens such as Salmonellae have mechanisms to resist these killing mechanisms that are environmentally regulated. The genes encoding these mechanisms are the subject of this grant. They include the virulence regulators PhoP/PhoQ that respond to signals within host tissues and induce genes necessary for resistance to innate immune killing. These regulators are essential for human and animal virulence. PhoP/PhoQ regulate genes involved in surface remodeling of bacteria. These genes include those responsible for modification of the lipid and protein components of the outer membrane. This grant proposes to define the mechanism by which these modifications are generated and the role of surface remodeling in bacterial virulence. The specific aims of this proposal are to define the genes involved in lipid A modification and the effects of these modifications on bacterial virulence and host cell recognition of lipid A. In addition a variety of genomic and proteomic techniques will be used to fully define the genes regulated by PhoP/PhoQ to better understand the coordinately regulated response of bacteria to host colonization.

Grant: 2R01AI031431-09A1
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: 1991/09/30-2005/03/31

DESCRIPTION (provided by the applicant): The long-term goal of this research 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 which humoral and cellular immune mechanisms stimulated by ehrlichiae are effective in the clearance of ehrlichiae. HME is a life-threatening tick-borne infection associated with adult respiratory distress syndrome, meningitis, and shock in immunocompetent patients, overwhelming infection in immunocompromised patients, and a fatality rate of 2.7 percent. More than 2,200 cases have been diagnosed with laboratory confirmation, and the incidence is 1,000 cases per 1,000,000 population in tick-exposed rural populations. The specific aims test the hypothesis that the immunodominant, surface-exposed p28 antigens stimulate protective immunity by antibodies and cellular mechanisms and determine the importance and mechanisms) of antibodies and cellular mechanisms of protective immunity in mouse models of HME. The research design includes sequencing of the loci of the p28 multigene families of major immunodominant surface proteins of Ehrlichia muris and a related ehrlichia (IOE) DNA and recombinant protein vaccines in the E. muris mouse model and the highly pathogenic IOE-C57BL/6 mouse model. Humoral immunity will be passive polyclonal and monoclonal antibodies to the conserved and variable regions of p28 families in IOE-infected mice including Fc-receptor knockout mice. Opsonization will be investigated in murine macrophages in vitro with E. muris and specific polyclonal and monoclonal antibodies. Cellular immune mechanisms will be elucidated using gene knockout mice (MHC Class I, MHC Class II, delta T-cell receptor, IFN-gamma, perforin, iNOS, and TNF-alpha receptor) and TNF-alpha depleted mice, immunohistochemical and flow cytometric analyses of the cell subsets and their cytokine profiles, adoptive transfer of T-lymphocyte subsets including T-cell clones in the outstanding new mouse model of HME.

Grant: 2R01AI032943-09
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: STEPHENS, RICHARD S PHD
Title: MICROBIOLOGY OF CHLAMYDIA TRACHOMATIS ATTACHMENT
Institution: UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA
Project Period: 1992/07/01-2005/12/31

DESCRIPTION (Adapted from the Applicant's Abstract): Chlamydia trachomatis is an obligate intracellular bacterial pathogen that is the cause of a wide spectrum of human diseases, including sexually transmitted diseases and blinding trachoma. Chlamydiae infect mammalian cells by attachment, endocytosis and inhibition of lysosomal fusion with endosomes containing chlamydiae. The target host cell in vivo is typically the columnar epithelial cell, and the primary mode of entry of chlamydiae into these 'non-professional phagocytic' cells is thought to be receptor-mediated endocytosis. Immunopathology caused from repeated and persistent infection causes the most severe disease outcomes; however, little is known about the molecular mechanism of chlamydial infection of host cells. The long-term objective is to understand chlamydial pathogenesis and virulence in the context of the interaction of chlamydiae with their host cells. This will yield important fundamental information for a) understanding mechanisms of infection, b) mediators of virulence and c) the development of new approaches for intervention. The specific aims of this application will be to advance our studies to define molecular and biochemical mechanisms involved in chlamydial-specific interactions with mammalian host cells. The aims are derived from our data that demonstrate a novel and essential role for glycosaminoglycan mediated chlamydial invasion of eukaryotic cells. The hypothesis is that chlamydiae invade mammalian host cells by a heparan sulfate-like ligand and elicit responses by the host cell which have consequences for pathogenesis. The significance of these studies is an understanding of fundamental mechanisms of chlamydial pathogenesis and virulence as an active interplay between chlamydia and its host cell. The specific aims are: 1) Molecular characterization of the heparan sulfate-like invasin, 2) Identify and characterize the mammalian host cell chlamydial receptor, 3) Characterize the modification of host cell signal-transduction and regulatory pathways by chlamydiae, and 4) Test the protein expression and significance of chlamydia-induced changes in host cell gene transcription.

Grant: 2R01AI034238-09

Program Director: SIZEMORE, CHRISTINE F.

Principal Investigator: HOPEWELL, PHILIP C MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC

Title: Population Based Molecular Epidemiology of TB

Institution: UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA

Project Period: 1993/04/01-2006/07/31

DESCRIPTION (provided by applicant): Since January 1991 we have been using genotyping of *Mycobacterium tuberculosis* together with conventional epidemiological approaches to elucidate the distribution and dynamics of tuberculosis in San Francisco. During this time we have refined and validated molecular epidemiological methods and applied these methods in a systematic series of studies that have been used to guide interventions tailored to the prevailing epidemiological circumstances. This study will extend our previous population-based, molecular epidemiologic studies of tuberculosis in support of the broad objective of eliminating tuberculosis in San Francisco that is caused by the transmission of *Mycobacterium tuberculosis* in San Francisco. This objective can be measured only by long-term application of molecular epidemiological methods. In addition, we propose to contribute to this objective by utilizing our detailed understanding of the dynamics of tuberculosis in San Francisco to examine genetic factors in both host and microbe that are associated with transmission of *M. tuberculosis* and progression of tuberculosis infection to clinical tuberculosis. In the proposed studies we will be combining state-of-the-art molecular epidemiology with recent advances in molecular biology, genomics, and computational biology in the setting of an effective tuberculosis control program to address some of the major current impediments to the elimination of the disease. The specific aims have been divided into four closely related components, intended to examine the interrelationships between clinical and epidemiological features of tuberculosis and human host and microbial genetic events in a setting wherein findings can be translated quickly to tuberculosis control efforts. The specific aims are divided as follows: 1) Identification and evaluation of tuberculosis control strategies; 2) Quantification of exposure and transmission; 3) Identification of host gene expression responses that distinguish susceptible and resistant persons; 4) Identification of mycobacterial factors associated with various outcomes following exposure to infectious tuberculosis. The components of these aims are all related to elucidating the factors related to transmission of *M. tuberculosis* and directed toward providing the scientific basis for measures designed to prevent transmission.

Grant: 2R01AI035237-07A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: GROS, PHILIPPE PHD
Title: NRAMP1 IN MACROPHAGE DEFENCES AGAINST INFECTIONS
Institution: MC GILL UNIVERSITY MONTREAL, PQ
Project Period: 1993/09/30-2004/07/31

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 antibiotics resistance. 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 investigators have 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 also associated with susceptibility to Mycobacterial infections 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, they propose that Nramp1 functions as a divalent cation efflux pump at the phagosomal membrane to suppress bacterial replication. The current proposal has four major goals. The first, is to understand how Nramp1 delivery affects the physiological properties of the phagosome including maturation, acidification, and bactericidal activity of macrophages and neutrophils. The second, is to identify the substrate and mechanism of transport of Nramp1 at the phagosomal membrane. The third is to identify protein determinants responsible for Nramp1 targeting to the lysosome and residues essential for substrate binding and transport. The fourth is to map new mouse loci that affect, in an Nramp1-independent fashion, host resistance to infection with clinically relevant Mycobacteria. Together, these studies should clarify the role and mechanism of action of Nramp1 in phagocytes anti-microbial defenses, which may in turn suggest new avenues for intervention in infectious diseases.

Grant: 2R01AI036596-06A2
Program Director: LAUGHON, BARBARA E.
Principal Investigator: HAMANN, MARK T
Title: ANTIAIDS AGENTS FROM MARINE ORGANISMS
Institution: UNIVERSITY OF MISSISSIPPI UNIVERSITY, MS
Project Period: 1995/04/01-2004/05/31

DESCRIPTION: Natural product research during the last few decades has yielded thousands of novel, bioactive organic compounds from the marine environment. However, little has been done to explore the application of marine natural products for the treatment of the AIDS opportunistic infections *Mycobacterium* sp. The primary goal of this project is the gram scale isolation of those marine structural classes identified as having significant activity against *Mycobacterium* in vitro. The natural product scaffolds will be utilized for biological evaluation, structural modifications and lead optimization. The marine natural products of interest include: manzamine alkaloids; uranidine alkaloids; C19 hydroxy steroids; tetrabromo spirocyclohexadienylisoxazoles; scalarin sesterterpenoids and shikimate-sesquiterpenes. The isolation and structure determination of both the major and minor natural product constituents of these marine structural classes will be completed. Pure natural, semisynthetic and biotransformation products from these six unique structural classes will be evaluated for biological activity at the Tuberculosis Antimicrobial Acquisition & Coordinating Facilities (TAACF). Lead optimization studies of the active scaffolds isolated in 50mg or greater quantities (1 gram target isolation) will be completed using combinatorial/parallel synthesis and microbial transformations (including combinatorial). The marine natural products will be purified using preparative and semi-preparative high pressure liquid chromatography (HPLC), independently and interfaced with NMR and FTMS. The chemical structures of biologically active products will be determined with the use of 2D NMR and FTMS. Structure activity relationships (SAR) will be completed using the biological results generated from natural, semisynthetic and bioconversion products. In addition the microbial metabolism studies will provide valuable information regarding the metabolic fate of these lead marine derived anti-TB structural classes. Optimized marine natural product derived leads will be scaled-up for in vivo anti-TB assays in mice. Tuberculosis is an extremely serious disease infecting an estimated one-third of the world's population. The rapid spread of drug-resistant strains of this bacillus in the last several years has created an urgent need for novel therapeutic agents with new modes of action to counter this impending threat. The six marine-derived structural classes described in this proposal will undergo thorough preclinical evaluation to determine their potential as drug leads for the treatment of TB.

Grant: 2R01AI036901-06
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: NICHOLAS, ROBERT A
Title: Penicillin-resistant *Neisseria gonorrhoeae* (CMRNG)
Institution: UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC
HILL
Project Period: 1996/04/01-2006/05/31

Antibiotic resistance in *Neisseria gonorrhoeae* remains a very important problem. Penicillin and tetracycline, which were once the antibiotics of choice for treatment of gonococcal infections, are no longer used due to the preponderance of strains resistant to these agents. Resistance to currently recommended antibiotics is also increasing. My laboratory is interested in the mechanisms of chromosomally-mediated antibiotic resistance in the gonococcus, especially those that promote high- level resistance and subsequent treatment failure. Intermediate- level chromosomally-mediated resistance to penicillin and tetracycline is due to three resistance loci. These include the *penA* gene encoding altered forms of penicillin-binding protein 2 (PBP 2), the *mtr* loci conferring resistance to hydrophobic agents, and the *penB* gene, which decreases outer membrane permeability. The genes involved in mediating high-level penicillin resistance, however, have been difficult to identify. Our work during the last funding period has identified two resistance genes, *ponA* and *penC*, which together mediate high- level penicillin resistance, and a third gene, *tetGC*, which confers high-level tetracycline resistance. This proposal outlines experiments to clone and characterize the *penC* and *tetGC* genes and to elucidate the mechanisms by which they increase resistance. In addition, we propose experiments that follow up on our structure/function studies of the *penB* gene product, porin IB, to understand how mutations in this protein increase both penicillin and tetracycline resistance. We also propose studies to complete our work on the crystal structure of penicillin- binding protein 2 (PBP 2), an essential penicillin target, and several mutant forms that display a lower affinity for beta- lactam antibiotics. In addition, we will engage in new structural studies of wild-type and mutant forms of porin IB to explicate in molecular detail how mutations in this protein decrease antibiotic permeability. The combination of genetic, biochemical, biophysical, and structural approaches outlined in this proposal will provide important insight into the mechanisms by which this important human pathogen becomes resistant to antibiotics.

Grant: 2R01AI037454-06A1

Program Director: NEAR, KAREN A.

Principal Investigator: ANTONY, VEENA B MBBS
PHYSIOLOGY:PHYSIOLOGY
UNSPEC

Title: MONOCYTE RECRUITMENT IN PLEUROPULMONARY TB IN AIDS

Institution: INDIANA UNIV-PURDUE UNIV AT INDIANAPOLIS, IN
INDIANAPOLIS

Project Period: 1996/02/01-2006/01/31

DESCRIPTION: Tuberculosis is endemic among patients with AIDS and follows an aggressive course with poor localization of mycobacteria into granuloma and widespread infection. Granulomas are monocyte rich collections of cells which derive from the circulating peripheral blood monocyte (PBMC). Monocytes are recruited to the site of tuberculous infection by the interaction of C-C chemokines, (mainly monocyte chemoattractant protein-1, MCP- 1) and monocyte expression of the CCR2 receptor. It is the hypothesis of this proposal that recruitment and retention of the monocyte at the site of tuberculous infection is predicated not only on local release of MCP-1 but on the expression of CCR2 receptor on PBMC. CD4 depletion alters monocyte recruitment and retention by altering the in vivo expression of CCR2 on PBMC and macrophages. This inhibitory effect of CD4 depletion is due to the relative imbalance between Th1 and Th2 cytokines. We have developed a model of pleural tuberculosis in CD4 -/- and CD4 +/+ mice to evaluate the Th1/Th2 regulation of the CCR2 receptor on PBMC. We will evaluate our hypothesis in our model of pleural tuberculosis in vivo as well as in vitro in PBMC and elicited pleural macrophages (PM). Our specific aims are 1) To determine the in vivo monocyte influx, granuloma formation, mycobacterial clearance and mortality utilizing our model of pleural tuberculosis in a CD4 knockout mouse model, CD4 -/- mice and control, wild type CD4 +1+/- mice. 2) To determine the in vivo effect of CD4 depletion on the compartmentalized and peripheral expression of CCR2 receptor on PBMC and PM and the regulatory role of Th1 (IL2, IL-12) and Th2 (IL-10, IL-4) cytokines in CD4 -/- and CD4 +/+ mice with pleural tuberculosis. 3) To determine the in vitro molecular and cellular regulation of CCR2 expression in PBMC and PM in the presence of Th1 and Th2 cytokines and 4) To determine the role of tubercle bacilli stimulated pleural mesothelial cell derived cytokines in the regulation of CCR2 receptor on peripheral blood monocytes (PBMC) and pleural macrophages (PM) in vivo and in vitro. Understanding the mechanism of regulation of CCR2 receptor by Th1 and Th2 cytokines may help us discern the pathophysiology of pleuro-pulmonary tuberculosis seen in patients with AIDS and may help develop therapeutic modalities that augment host-defense responses.

Grant: 2R01AI037601-07A1
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: 1995/04/01-2005/12/31

DESCRIPTION (Adapted from the Applicant's Abstract): *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 Dr. Leong's group has focused on *Borreliae* recognition of two classes of host cell molecules: (1) glycosaminoglycans (GAGs); and (2) integrins and their associated proteins. For *B. burgdorferi*, they have 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 Dr. Leong's 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, in contrast to *B. burgdorferi*, *B. hermsii* bound and activated resting platelets. The platelet activation activity is apparently mediated by the integrin-associated platelet-signaling molecule CD9. Dr. Leong speculates that prior to the development of an antibody response, attachment of relapsing fever spirochetes to the vessel wall, either directly via GAGs or indirectly, via activated and adherent platelets, could diminish the clearance of bacteria from the bloodstream by the reticuloendothelial system. Continued replication by these adherent bacteria would result in high level bacterial seeding of the bloodstream. Interaction of spirochetes with platelets could also contribute to thrombocytopenia, a common manifestations of relapsing fever.

Grant: 2R01AI037657-05
Program Director: LANG, DENNIS R
Principal Investigator: TWETEN, RODNEY K PHD
Title: Pore Formation by Cholesterol Dependent Cytolysins
Institution: UNIVERSITY OF OKLAHOMA HLTH SCIENCES CTR OKLAHOMA CITY, OK
Project Period: 1997/04/01-2006/02/28

DESCRIPTION (provided by the applicant): Perfringolysin O (PFO), a cytolysin (Mr 54,000) produced and secreted by *Clostridium perfringens*, belongs to a family of related cytolysins now termed the cholesterol-dependent cytolysins (CDCs) and is produced by a variety of Gram positive pathogenic bacterial species. PFO typifies the CDCs, with a hydrophilic primary structure that ultimately forms a cytolytic membrane complex. After binding to the target membrane, PFO monomers oligomerize into supramolecular complexes and lyse the cell. During the current grant period, we identified the regions of PFO that form the aqueous-membrane interface and determined that each monomer inserted two B-hairpins into the bilayer to form the B-barrel of the pore. We also found that PFO forms a prepore complex prior to the insertion of these domains. The studies herein are designed to further our understanding of the mechanism by which these intriguing toxins alter their structure and interact with one another and the membrane surface as they make the transition from a soluble monomer to a membrane-bound oligomeric complex. The specific aims of this proposal are to: 1) Determine the topography of PFO relative to the membrane and identify intramolecular conformational changes at different stages of pore formation. 2) Elucidate the interactions between transmembrane B-hairpins in the oligomer. 3) Identify the PFO residues involved in subunit-subunit interactions. 4) Identify the nature of the intermedilysin receptor. Aim 1 will be accomplished by the use of fluorescence resonance energy transfer (FRET) to measure distances from various points in the PFO structure to the membrane surface at different stages of its membrane penetration. Aim 2 will be accomplished by characterizing the ability of native toxin to induce the insertion of the transmembrane B-hairpins (TMHs) of PFO mutants that alone can form an oligomeric prepore, but cannot insert their TMHs. In aim 3 the monomer-monomer interfaces of PFO in the membrane-bound oligomeric complex will be revealed by the lack of accessibility to aqueous and membrane-restricted collisional quenchers of a fluorescent probe that will be placed at various locations on the surface of the monomer in cysteine-substituted derivatives of PFO. The location of the residue at an interface will be confirmed by site-specific crosslinking. Finally, we will investigate the intriguing property of intermedilysin, a member of the CDC family, which restricts its erythrocyte specificity to human erythrocytes, in contrast to all other known CDCs. We will identify and characterize the receptor for intermedilysin by a combination of receptor blots and affinity purification methods.

Grant: 2R01AI037844-06A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: NORTH, ROBERT J PHD
PATHOLOGY:EXPERIMENT
Title: IMMUNOLOGIC CONSEQUENCES OF M. TUBERCULOSIS VIRULENCE
Institution: TRUDEAU INSTITUTE, INC. SARANAC LAKE, NY
Project Period: 1995/06/01-2006/02/28

DESCRIPTION (Adapted from the Applicant's Abstract): The proposed experiments are designed to investigate the limited protective value of the primary and secondary immune responses to airborne infection with virulent *M. tuberculosis* (Mtb) in mice. The secondary response will be studied in mice that possess a state of immunological memory as a result of having been infected with avirulent or virulent live Mtb and later treated with chemotherapy to essentially abolish infection. Vaccinated and unvaccinated mice will be infected with virulent Mtb by aerosol and 5 mice sacrificed at progressive times of infection to follow the progression and subsequent immunological control of infection. Additional mice will be sacrificed to determine the kinetics of production in the draining tracheobronchial lymph node of Mtb-specific CD4 Th1 cells and CD8 T cells, identified and enumerated by their ability to secrete IFN γ in response to Ag stimulation in the ELISPOT assay. The appearance of these T cells in the draining nodes will be compared with their appearance in blood and at sites in infection in the lungs. It will be determined, with the RNase protection assay and Western blotting, whether the onset of expression of immunity in the lung is associated with transcriptional activation of genes for Th1 cytokines needed for the mediation of immunity, and if iNOS needed for the expression of immunity by macrophages at infectious foci. The prediction that neither the primary, nor the secondary immune response will be capable of preventing the establishment of infection even with very small numbers of virulent Mtb, or even with a virulent Mtb, because of an intrinsic delay before immunity can be expressed in the lungs will be tested. The prediction that, once expressed, immunity will be unable to resolve even very low levels of infection, but will cause infection to become stationary, will also be tested by challenging mice with small numbers of Mtb, and by later reducing the number of Mtb in lesions with chemotherapy as immunity is being expressed. Whether the delay in expression of immunity is caused by a delay in the production of protective T cells, or a delay in the development of conditions at infectious foci necessary for the extravasation of T cells at these sites will be investigated by adoptive transfer studies. The inability of the secondary immune response to enable genetically susceptible mice to stop lung infection from progressing will be investigated. The possibility that genetically susceptible mice, will be incapable of stopping lethal regrowth of a greatly reduced level of lung infection following chemotherapy will also be investigated.

Grant: 2R01AI037901-06A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: HUSSON, ROBERT N MD
Title: Characterization of Mycobacterial Sigma Factors
Institution: CHILDREN'S HOSPITAL (BOSTON) BOSTON, MA
Project Period: 1995/06/01-2005/02/28

DESCRIPTION (provided by the applicant): The long-term goal of this research is to investigate the role of the alternative sigma factors SigD, SigE, SigH and SigM in the regulation of Mycobacterium tuberculosis gene expression and virulence. The underlying hypothesis of this research is that extracytoplasmic function (ECF) sigma factors of M tuberculosis play an important role in the regulation of gene expression during the interaction of this organism with the host during infection, and that this regulation is important for the virulence of this organism. This proposal has four complementary specific aims. The first aim is to complete construction of sigma factor mutant strains of M tuberculosis and to compare the survival of these mutants vs. the parental strain H37Rv following in vitro oxidative and nitrosative stresses that may be relevant to infection. The second aim is to compare the virulence of these mutants vs. H37Rv in macrophage and mouse models of infection. Mutant strains will be complemented to verify that any phenotypes observed in these experiments result from the disruption of the sigma factor gene that was mutated. The third aim is to investigate the role of these sigma factors in regulating M tuberculosis gene expression, focusing on the use of microarray technology, supplemented with bioinformatic and traditional molecular genetic methods. The fourth aim is to investigate the role of specific sigma factor-regulated genes in M tuberculosis virulence, by examining their expression and by constructing and testing M tuberculosis strains with mutations in these genes. These investigations are expected to identify regulatory networks and specific genes that are important in M tuberculosis virulence. This research thus has the potential to generate new insights into the pathogenesis of M tuberculosis infection, and may provide the basis for new approaches to the control of tuberculosis.

Grant: 2R01AI038417-06
Program Director: KLEIN, DAVID L
Principal Investigator: MILLER, JEFF F
Title: BORDETELLA VIRULENCE REGULON IN VITRO AND IN VIVO
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 1995/07/01-2006/03/31

DESCRIPTION (Adapted from applicant's abstract): The BvgAS signal transduction system coordinately regulates genes and operons involved in the Bordetella infectious cycle. Recent results indicate that the Bordetella virulence regulation is more complex, and interesting, than we had previously imagined. As opposed to mediating a biphasic transition, BvgAS appears to control a spectrum of gene expression states. We have also discovered the BvgAS regulates type III secretion, which functions at least in part to modulate host immunity. In the course of studying Bvg AS, type III secretion, and other virulence associated factors and phenotypes, we have developed genetic methods for manipulating Bordetella genomes and a variety of animal models for studying infection and the infectious cycles. The experiments outlined in this application are a direct extension of our most interesting observations to date. They represent a desire to understand the biology of the bordetellae from the perspectives of virulence gene regulation and the evolution of bacterial pathogenesis. In Aim 1 we will conduct a genetic and phylogenetic analysis of signal responsiveness by the BvgAS virulence control system. Bvg-regulated loci display markedly diverse patterns of gene expression which we hypothesize reflects a requirement for differential gene expression throughout the infectious cycle. We will characterize relationships between temporally and spatially controlled transcription levels of Bvg-regulated genes, determine if key characteristics of Bvg-mediated signaling are phylogenetically conserved, and identify sequences that determine signal responsiveness. In Aim#2, transcription of Bvg-regulated genes will be measured in vivo and requirements for differential gene expression during the infectious cycle will be examined. We will test the hypothesis that differential gene expression at different sites in the respiratory tract results in adaptation to specific niches and facilitates both infection and transmission. Aim #3 will focus on the regulation, phylogenetic conservation and comparative function of type III secretion by Bordetella subspecies. In Aim 4, we will use high density DNA microarrays to identify the entire complement of Bvg-regulated genes and investigate their functions in animal models of infection and transmission. We will test the hypothesis that the Bvg regulon is multiphasic by conducting a genome-wide expression analysis. Differences in expression profiles between B. pertussis, B. parapertussis and B. bronchiseptica will also be determined in an effort to understand subspecies-specific differences in Bordetella-host interactions.

Grant: 2R01AI038459-05A1
Program Director: LANG, DENNIS R
Principal Investigator: TOMPKINS, LUCY S MD INTERNAL
MED:INFECTIOUS DISEASE
Title: GENETIC AND CELLULAR BASIS OF H. PYLORI PATHOGENESIS
Institution: STANFORD UNIVERSITY STANFORD, CA
Project Period: 1996/05/01-2006/02/28

DESCRIPTION (Adapted from the Applicant's Abstract): The goal of the proposed research is to understand better the interaction between *H. pylori* and its human host. *H. pylori* is the causative agent of gastritis and ulcer disease, and infection is an important risk factor for the development of gastric cancer. In previous studies, these investigators have shown that *H. pylori* CagA is transported into the host cell, and is then phosphorylated on tyrosine residues by host cell kinase activity. The insertion of CagA into host cells and its subsequent phosphorylation is associated with reorganization of the host cell cytoskeleton, as well as a dramatic change in host cell morphology. The investigators propose to examine the insertion of CagA into host cells and the sequence of events following CagA insertion. In addition, they propose to alter the structure of CagA by mutagenesis to determine the role of specific protein domains in bringing about cellular changes. In a second phase of work, these investigators have obtained DNA arrays of the *H. pylori* genome and host gene arrays of both human and mouse genes. They propose to use the *H. pylori* DNA array to examine the genotype of clinical isolates from well-defined epidemiological studies to determine whether particular genes or groups of genes are associated with discrete clinical syndromes like ulcer disease or malignancy. The major thrust of the proposed work will focus on the use of DNA arrays to follow gene transcription of both bacterial and host genes during infection of polarized cultures of human epithelial cells. They hypothesize that this will permit the identification of new classes of virulence genes. The sequence of the host response as measured by gene transcription is suggested to permit better understanding of the host cell pathways that are exploited by the bacteria during infection. Finally, a method called microarray transposon tagged *H. pylori* (MATT) will be used, which permits the identification of specific classes of mutation from selective environments, including infected cell cultures and animals.

Grant: 2R01AI038894-06

Program Director: SAVARESE, BARBARA M.

Principal Investigator: RADOLF, JUSTIN D MD CLINICAL MEDICAL SCIENCES, OTHER

Title: Cutaneous Immune Response in Early Syphilis

Institution: UNIVERSITY OF CONNECTICUT SCH OF FARMINGTON, CT
MED/DNT

Project Period: 1996/04/01-2006/05/31

DESCRIPTION (Applicant's Abstract): Venereal syphilis is a chronic inflammatory disorder driven by the persistence of its etiologic agent *Treponema pallidum*. Research in this proposal is based on the premise that the local (i.e., tissue-based) cellular immune responses to *T. pallidum* have two distinct, yet interrelated, consequences of fundamental importance to syphilis pathogenesis. They cause the tissue damage which ultimately gives rise to clinical manifestations, and they are primarily responsible for the clearance of bacteria, a prerequisite for lesion resolution. Human skin is the primary focus of our efforts to elucidate these processes because (a) it is the major target organ of early syphilitic infection, (b) it is easily accessible to in vivo experimentation, and (c) there exists a wealth of reagents and information concerning its immune-related functions. During the prior funding interval, we have made considerable progress in characterizing the cellular infiltrates in secondary syphilis lesions and in delineating the ontogeny of the cutaneous response engendered by the syphilis spirochete. A unifying theme of this work has been the acquisition of considerable evidence, using a combination of in vitro and in vivo approaches, to support our primary hypothesis that the proinflammatory properties of treponemal lipoproteins are the primary triggers of innate immune mechanisms in early syphilis. More recently, we have shown that by recruiting a cellular infiltrate rich in antigen presenting cells, particularly dendritic cells, and memory/effector T cells, the innate immune processes induced by these lipid-modified polypeptides set the stage for the adaptive (i.e., specific) immune responses to the bacterium. One important outcome of these findings is the recognition that the cutaneous responses under investigation relate to primary as well as to secondary syphilis. In this competitive renewal application, we will extend this conceptual framework by further characterizing the in vivo biological responses to treponemal lipoproteins/lipopeptides (Aim One); by further characterizing the cutaneous immune response to *T. pallidum* in secondary syphilis lesions (Aim Two); by using in vitro/ex vivo approaches to examine dendritic and T cell responses to *T. pallidum* and treponemal proteins (Aim Three); and by examining our hypothesis that treponemal lipoproteins activate macrophages following uptake and degradation within the phagosomal vacuoles of macrophages (Aim Four). This work will result in an enhanced appreciation of the role of local cellular responses in syphilis pathogenesis and will provide a necessary underpinning for the eventual development of a safe and effective syphilis vaccine.

Grant: 2R01AI038897-06
Program Director: MILLER, MARISSA A.
Principal Investigator: SCHNEEWIND, OLAF MD
Title: SURFACE PROTEIN ANCHORING IN GRAM-POSITIVE BACTERIA
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 1996/02/01-2001/06/30

DESCRIPTION (Adapted from the Applicant's Abstract): Human infections caused by Gram-positive bacteria present a serious therapeutic challenge due to the appearance of antibiotic-resistant strains. Of particular concern is *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Enterococcus faecalis*, Gram-positive organisms that are the most common cause of bacterial infections in American hospitals. These nosocomial pathogens have developed resistance mechanisms to all known antibiotic regimens and the development of novel targets for antimicrobial therapy is urgently needed. Surface proteins of Gram-positive organisms fulfill many important functions during the pathogenesis of human infections. This proposal describes the mechanism for surface protein anchoring in Gram-positive bacteria, which may serve as a target for antibacterial therapy. Staphylococcal surface proteins harbor a C-terminal sorting signal that functions first to retain polypeptides within the secretory pathway. Retention is followed by cleavage of the sorting signal between the threonine (T) and the glycine (G) of the LPXTG motif. The carboxyl of threonine is subsequently amide linked to the free amino group of peptidoglycan crossbridges, thereby anchoring the C-terminal end of surface proteins to the staphylococcal cell wall. Sortase, a membrane anchored enzyme of *S. aureus*, catalyzes a transpeptidation reaction, capturing cleaved surface protein as a thioester intermediate at the active site sulfhydryl. Nucleophilic attack of the amino group of pentaglycine crossbridges resolves the thioester intermediate, resulting in cell wall anchored surface protein and in regeneration of enzyme sulfhydryl. The elements and enzymes of surface protein anchoring, i.e., the LPXTG motif, the amino groups of peptidoglycan as well as sortase, are conserved in Gram-positive bacteria. This, we propose that surface protein anchoring is a universal mechanism. To test this hypothesis, we will characterize sortase function in *S. aureus*, *E. faecalis* and *L. monocytogenes*. Further, we propose identification of the peptidoglycan substrate of the sortase reaction, using in vivo labeling techniques as well as biochemical characterization of sorting intermediates in *S. aureus*, *E. faecalis* and *L. monocytogenes*. A genetic screen for *S. aureus* mutants defective in the retention step of surface protein anchoring will identify missing components of the cell wall sorting machinery.

Grant: 2R01AI039454-05A1
Program Director: LAUGHON, BARBARA E.
Principal Investigator: WEISS, LOUIS M MD MEDICINE
Title: TOXOPLASMA ENCEPHALITIS IN AIDS:BRADYZOITE REGULATION
Institution: YESHIVA UNIVERSITY NEW YORK, NY
Project Period: 1996/04/01-2004/12/31

DESCRIPTION: *Toxoplasma gondii* is a ubiquitous Apicomplexan protozoan parasite of mammals and birds, which is responsible for several important clinical syndromes in humans. The predilection of this parasite for the central nervous system (CNS) causing behavioral disorders and especially, fatal necrotizing encephalitis constitutes its major threat to patients with HIV infection. CNS toxoplasmosis ranks among the ten most commonly occurring opportunistic infections in AIDS patients, and may well be a greater direct cause of morbidity and mortality than other more common opportunistic infections. If acquired during pregnancy infection can result in the syndrome of congenital toxoplasmosis with attendant encephalitis, mental retardation, and chorioretinitis. In both conditions, reactivation of the latent encysted state of the organism (bradyzoite) to the active replicative form (tachyzoite) is associated with progression of disease and is directly implicated in the pathology that attends this infection. Despite major advances in our understanding of the cell biology of *T. gondii* as well as our ability to manipulate this parasite in vitro, very little is known about the developmental pathways and control mechanisms in the transition of tachyzoites to bradyzoites. Stress conditions are associated with the induction of bradyzoite development and in the last granting period we identified several heat shock proteins (hsps) associated with this differentiation event. BAG 1, a small heat shock protein (shHsp), is induced early during bradyzoite differentiation. This shHsp was cloned and a knockout constructed. The BAG1 knockout formed fewer cysts in vivo; suggesting that BAG1 was associated with the ability of *T. gondii* to form cysts. In addition, we identified a glycoprotein, CST1, that is also induced early during bradyzoite development. We now plan to focus our studies on the differentiation or developmental biology of *T. gondii* and define those factors that are involved in the interconversion of the active or acute stage to the latent or chronic stage of this disease. Thus, this proposal will entail the characterization of a unique early bradyzoite antigen CST1, definition of the function(s) of BAG1 (a shHsp) and the characterization of the stress response in *T. gondii*. Each of these specific aims will further our understanding of the early events in bradyzoite differentiation leading to chronic (latent) toxoplasmosis. Therapeutic modalities aimed at interdicting bradyzoite formation should eradicate this infection in the infected host, thereby eliminating bradyzoite reactivation which is the key element in the development of CNS toxoplasmosis in AIDS.

Grant: 2R01AI039557-05A1
Program Director: LANG, DENNIS R
Principal Investigator: FANG, FERRIC C MD CLINICAL MEDICAL
SCIENCES, OTHER
Title: NITRIC OXIDE CYTOTOXICITY IN SALMONELLOSIS
Institution: UNIVERSITY OF COLORADO DENVER/HSC DENVER, CO
AURORA
Project Period: 1997/06/01-2001/06/30

DESCRIPTION (provided by applicant): The focus of research in my laboratory is on host-pathogen interactions. Toward that end, we are studying how phagocytes inhibit or kill intracellular microbes using reactive oxygen species (ROS) and nitrogen species (RNS) produced by the NADPH phagocyte oxidase and inducible nitric oxide synthase (iNOS). The specific antimicrobial effector molecules, their targets, and mechanisms of resistance remain incompletely understood. Both the NADPH oxidase and iNOS are required for innate murine resistance to Salmonella infection. Preliminary studies suggest the hypothesis that direct interactions with intracellular free iron determine the antimicrobial actions of ROS and RNS, and regulate relevant stress responses. We propose a novel model in which intracellular free iron potentiates the antimicrobial actions of nitric oxide (NO) and its synergistic interactions with hydrogen peroxide (H₂O₂). Nitrosative stress induces the expression of iron-repressed proteins such as superoxide dismutase and the Hmp flavohemoprotein via direct NO-iron interactions, which in turn enhance microbial resistance to both ROS and RNS. The specific aims of this proposal are to test predictions of our experimental model by: [1] Comparing Salmonella gene regulation by nitric oxide and iron deprivation; [2] Assessing free intracellular iron as a determinant of susceptibility to ROS and RNS; [3] Performing mutagenesis of the Salmonella Hmp flavohemoprotein to identify domains involved in detoxification of ROS and RNS; [4] Determining the relationship between host and microbial intracellular iron availability and ROS/RNS-dependent antimicrobial activity. These aims will be achieved by a combination of genetic, biochemical and in vivo analyses. The results will have important implications for a molecular understanding of microbial pathogenesis as well as of NO-iron interactions in a variety of fundamental biological processes.

Grant: 2R01AI039558-06
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: STARNBACH, MICHAEL N PHD
Title: Cytotoxic T-cell Mediated Immunity to Chlamydia
Institution: HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA
Project Period: 1996/07/01-2006/05/31

DESCRIPTION (provided by the applicant): The obligate intracellular pathogen *Chlamydia trachomatis* is the most common cause of sexually transmitted disease in the developed world, causing both overt disease and infertility, and is also the leading cause of preventable blindness worldwide. Infection with *C. trachomatis* results in a specific immune response against the organism, and CD8+ cytotoxic T-lymphocytes (CTL) specific for *C. trachomatis* can be cultured from infected mice. Adoptive transfer of cultured CD8+ T-cells into infected mice reduces the number of organisms found in the spleens of these animals. The experiments in this proposal are designed to determine which *Chlamydia* gene products are recognized by these *Chlamydia* specific murine CD8+ T-cells. Three independent approaches will be used to identify these antigens. Once the *C. trachomatis* gene products responsible for priming the CD8+ T-cell response are identified, they will be introduced into vaccinia virus and tested for their ability to stimulate protective T-cells in a vaccine strategy. Because these antigens stimulate CD8+ T-cells, it suggests that they have access to the cytosol of host cells during the *C. trachomatis* developmental cycle. Experiments to characterize the developmental regulation and subcellular localization of these proteins will be carried out as a first step in understanding the role these proteins may play in *C. trachomatis* pathogenesis. Additional experiments will focus on the mechanism by which CD8+ T-cells protect against *Chlamydia* infection. Interferon-gamma (IFN-g) appears to be the primary effector mechanism used by these T-cells to protect against *C. trachomatis* infection. IFN-g release by CD8+ T-cells could protect mice against *C. trachomatis* infection by stimulating the antimicrobial activity of macrophages or by directly inhibiting the replication of *C. trachomatis*. Experiments to clarify the mechanism by which IFN-g produced by T-cells mediates protection will use radiation bone marrow chimeras in which either the hematopoietic cells (including macrophages) or the resident cells are unable to respond to IFN-g as a result of a disruption in the IFN-g receptor gene. A better understanding of the immune response to *C. trachomatis* and the development of a vaccine would have a pronounced effect on worldwide morbidity resulting from these infections.

Grant: 2R01AI039575-06
Program Director: BAKER, PHILLIP J.
Principal Investigator: PLANO, GREGORY V PHD
Title: Control of virulence protein export in *Yersinia pestis*
Institution: UNIVERSITY OF MIAMI-MEDICAL CORAL GABLES, FL
Project Period: 1996/06/01-2006/05/31

DESCRIPTION (provided by the applicant): *Yersinia pestis*, the etiologic agent of plague, uses a type III secretion system (TTSS) to translocate virulence proteins, termed Yops, into eukaryotic cells. Yop export is triggered by contact with a host cell in vivo or by growth in the absence of Ca^{2+} in vitro. The *Y. pestis* YopN, TyeA, SycN, and YscB proteins are required to prevent Yop secretion in the presence of Ca^{2+} and prior to contact with a eukaryotic cell. Previous studies have established that the SycN and YscB proteins function as specific chaperones for the secreted YopN protein. A complex of SycN and YscB, but not SycN or YscB alone, directly binds to an N-terminal domain of YopN. TyeA, on the other hand, binds to a C-terminal domain of YopN. This project will analyze the function of the YopN-TyeA-SycN-YscB complex in the regulation of Yop secretion. The control of virulence protein secretion is of broad interest because of the realization that numerous bacterial pathogens employ such "cell contact-dependent" delivery systems. The goals of this project are: (i) to define the function of the SycN/YscB chaperone complex in YopN secretion, YopN translocation and in the regulation of Yop secretion; (ii) to identify the mechanism by which the YopN-TyeA-SycN-YscB complex blocks secretion; and (iii) to identify the signal, sensors and signal transduction pathways that lead to the removal of the YopN-TyeA-SycN-YscB-dependent block in Yop secretion. The objectives of this project will be realized through the selection and analysis of specific *Y. pestis* mutants that fail to block Yop secretion, constitutively block Yop secretion, or effect specific protein-protein interactions within the YopN-TyeA-SycN-YscB complex. Immunoprecipitation experiments and yeast two- and three-hybrid analyses will be used to identify and characterize novel protein-protein interactions. Biochemical and biophysical analyses will be employed to determine the stoichiometry, conformation and binding constants of specific proteins or protein complexes. A *Y. pestis* chromosomal transposome insertion library will be screened to identify specific insertion mutants that are defective in Yop secretion or in the regulation of Yop secretion. These studies will provide insight into the role of the YopN-TyeA-SycN-YscB complex in the regulation of Yop secretion in *Y. pestis*.

Grant: 2R01AI039654-06
Program Director: LANG, DENNIS R
Principal Investigator: TAYLOR, RONALD K PHD OTHER AREAS
Title: Hierachy Within Environmental Regulons
Institution: DARTMOUTH COLLEGE HANOVER, NH
Project Period: 1996/06/01-2006/06/30

DESCRIPTION (provided by the applicant): Infection by bacterial pathogens continues to be a major health concern throughout the world. For example, during the past decade the incidence of cholera spread to more areas of the globe than had occurred in the previous 100 years. A better understanding of the molecular bases of pathogenesis may provide new ways to combat bacterial infection. The goal of this research is to discern the mechanisms by which physiological signals in the environment within the human host are converted to molecular interactions that govern the expression of virulence genes of the infecting bacterium. The model system to be analyzed is the *Vibrio cholerae* ToxR virulence regulon, for which a number of parameters that influence gene expression, as well as many of the regulators and target genes, are known and partially characterized. The target virulence genes include the tcp operon, toxT, and other "ToxR activated" genes present on the *Vibrio cholerae* TCP pathogenicity island, as well as the ctx operon present on a lysogenic bacteriophage. The regulators are encoded by genes distributed around the genome, including toxRS, aph4, aphB, hns, and crp, as well as the tcpPH and toxT genes present on the pathogenicity island. It has recently been determined that multiple regulators function at each target gene promoter. The current proposal focuses on a subset of target promoters and regulators to understand how these regulators function in an interaction with growth condition signals, the promoters, and with each other to control gene expression. In addition, the identity of additional target genes for which expression is influenced by the regulatory proteins that are not encoded within the *Vibrio* TCP pathogenicity island will help to better understand the precise chemical and physical responses that are being converted into virulence gene expression mechanisms. Correlating virulence gene expression together with regulatory responses that modulate bacterial physiology represents a new approach that utilizes knowledge of the genome to further our understanding of the basis of virulence gene regulation. Finally, new approaches to monitor virulence gene expression both in vitro and in vivo will provide a means to correlate these two events and to identify additional genes involved in regulation. These experiments will likely reveal novel virulence factor genes that may prove to be useful vaccine or therapeutic targets. A further understanding of virulence gene expression will also help in the development of ways to modulate it either in vivo or in vitro for improved vaccine production or overproduction of virulence factors for structural analyses.

Grant: 2R01AI039657-06A1
Program Director: LANG, DENNIS R
Principal Investigator: COVER, TIMOTHY L MD
Title: Structure-function analysis of H. pylori VacA
Institution: VANDERBILT UNIVERSITY NASHVILLE, TN
Project Period: 1996/05/01-2006/07/31

DESCRIPTION (provided by the applicant): Colonization of the human gastric mucosa by *Helicobacter pylori* is associated with an increased risk for development of peptic ulcer disease and distal gastric adenocarcinoma. Studies in a mouse model for H. pylori infection indicate that expression of a toxin (VacA) enhances the capacity of H. pylori to colonize the stomach, and immunization of mice with VacA results in protective immunity. VacA contributes to gastric mucosal damage, and analysis of vacA alleles in H. pylori isolates from humans suggests that VacA plays a role in the pathogenesis of peptic ulcer disease. The effects of VacA on eukaryotic cells include vacuolation, altered trafficking within the endocytic pathway, membrane channel formation, and apoptosis. The VacA mechanism of action remains incompletely understood. Based on our preliminary studies, we hypothesize that the mature VacA toxin can be divided into three functional domains: (i) an N-terminal hydrophobic region (amino acids 1-32) involved in membrane insertion, transmembrane protein dimerization, and membrane channel formation; (ii) an N-terminal region (amino acids 33-422) that is required for intracellular toxin activities (cell vacuolation and apoptosis); and (iii) a C-terminal domain (amino acids 423-821) involved in binding of VacA to eukaryotic cells. This proposal outlines plans for in-depth structure-function analysis of these three domains. We will use multiple experimental approaches, including several mutagenesis strategies, assays of VacA channel activity, an in vitro system for analyzing peptide insertion into membranes, expression of recombinant VacA, a system for intracellular VacA expression, mapping of VacA structure with recombinant anti-VacA antibodies, and use of a mouse model to examine the functions of VacA in vivo. These studies should result in a better understanding of VacA structure and function, and insights into the VacA mechanism of action. Ultimately, these studies may lead to advances in the treatment or prevention of H. pylori-associated human diseases.

Grant: 2R01AI040567-06A1
Program Director: KORPELA, JUKKA K.
Principal Investigator: NEMANI, PRASADARAO V PHD
Title: Brain Endothelial Receptor for E. coli
Institution: CHILDREN'S HOSPITAL LOS ANGELES LOS ANGELES, CA
Project Period: 1997/01/01-2006/04/30

DESCRIPTION (provided by applicant): Neonatal Escherichia coli meningitis continues to be a diagnostic and treatment challenge despite the availability of active antibiotics. Investigations on the understanding of the pathogenesis and pathophysiology are needed to develop novel strategies to prevent the high morbidity and mortality associated with this disease. OmpA of E. coli is one of the major factors responsible for E. coli traversal across the blood-brain barrier that constitutes a lining of brain microvascular endothelial cells (BMEC). Ecgp, a novel blood-brain barrier specific glycoprotein interacts with OmpA to induce actin rearrangement for subsequent penetration into the central nervous system, has been identified and characterized. Recombinant Ecgp significantly blocked the E. coli traversal across the blood-brain barrier indicating the biological relevance of OmpA-Ecgp interaction in the development of this disease. Ecgp itself is phosphorylated to interact with two BMEC cytoplasmic signaling proteins, FAK and Vav2. in response to E coli infection. The identification of ligand binding and signaling domains of Ecgp will help elucidate the molecular mechanisms of OmpA-Ecgp interaction and its importance in the pathogenesis and pathophysiology of E. coli meningitis. Hypothesis: OmpA-Ecgp interaction is a key step that signals BMEC for E. coli traversal across the blood-brain barrier to cause meningitis. The following are the specific aims to test this hypothesis: 1. Characterize the antigenic structure of Ecgp using monoclonal antibodies against Ecgp that block E. coli invasion of HBMEC both in vitro and in the newborn rat model of hematogenous meningitis. 2. Identify the minimum structures of the Ecgp extracellular domains that interact with OmpA responsible for E. coli invasion of HBMEC by molecular modeling and site directed mutagenesis of Ecgp. 3. Identify the intracellular motifs of Ecgp that interact with the HBMEC cytoplasmic proteins, FAK and Vav2.

Grant: 2R01AI041231-06
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: EKO, FRANCIS O PHD
Title: INDUCTION OF PROTECTIVE IMMUNITY AGAINST CHLAMYDIA
Institution: MOREHOUSE SCHOOL OF MEDICINE ATLANTA, GA
Project Period: 1996/09/01-2006/06/30

DESCRIPTION (provided by the applicant): Genital infection by the obligate intracellular pathogen, Chlamydia trachomatis, is the most common bacterial sexually transmitted disease (STD) in the United States, with four million reported annual cases that cost over \$2 billion. Of major pathophysiological significance is the propensity for cervical infection in women to spread into the upper genital tract, provoking serious complications such as pelvic inflammatory disease, fallopian tube scarring, ectopic pregnancy and infertility. Also, the frequently asymptomatic infections do cause severe irreversible complications to be the first evidence of an infection. There are concerns that genital chlamydial disease, like certain other STDs, such as AIDS and gonococcal disease, may pose a serious threat to human reproduction, well-being and healthcare costs. Current control and prevention strategies target frequent screening for early detection and treatment, and development of vaccines as the priority. The search for a chlamydial vaccine has led to extensive research to define the crucial immune effectors in anti-chlamydial immunity, identify antigens that elicit protective immunity, and design effective methods of vaccine delivery. Our research has been focused on identifying the relevant immune parameters in chlamydial immunity and elucidating the mechanism(s) of intraepithelial inhibition of chlamydiae. Our findings and reports by others have culminated in a new paradigm for designing vaccines against Chlamydia based on the induction of local mucosal TH1 response. The major challenge at this stage is to select an appropriate immunogen(s) and design an effective delivery system, to induce high levels of local genital mucosal Th1 response to maintain long-term immunity. Accordingly, this proposal uses immunological, genetic engineering, molecular, cellular and biochemical techniques to investigate the central hypothesis that protective anti-chlamydial immunity will be established if immunogenic chlamydial antigen(s) are effectively delivered to induce high frequency of specific Th1 cells in the genital mucosa. Specific studies planned will use genetically engineered and wild type mice to: (a) investigate the efficacy of genetically designed recombinant multi-subunit vaccines composed of mucosal bacterial ghosts co-expressing multiple membrane proteins of C. trachomatis; (b) assess the therapeutic benefits of an immunotherapeutic cellular vaccine based on IL-10 gene-suppressed dendritic cells presenting antigens for inducing high frequency of specific Th1 response, as an alternative therapeutic vaccine for C. trachomatis; (c) identify the major mucosal inductive sites, antigen-presenting cells and other accessory cells crucial for Th1 activation; and (d) define the molecular and cellular elements regulating Th1 activation, trafficking and recruitment into the genital mucosa following effective cellular and subunit vaccination against C. trachomatis. Results from these studies will likely lead to the development of a reliable vaccine regimen against Chlamydia, which should have major implications for the genital, ocular, and lung infections and their complications.

Includes RPGs (Research Project Grants)
Excludes Clinical Trials

Grant: 2R01AI041735-04A2
Program Director: LAUGHON, BARBARA E.
Principal Investigator: WHITE, ARTHUR CLINTON MD
Title: Intestinal Cytokines in Cryptosporidiosis
Institution: BAYLOR COLLEGE OF MEDICINE HOUSTON, TX
Project Period: 1997/09/01-2006/07/31

DESCRIPTION (Provided by the applicant): *Cryptosporidium parvum* is a major cause of diarrhea worldwide, for which there is no reliable antiparasitic therapy. In immunocompetent individuals, *C. parvum* infection results in a self-limited diarrheal illness. By contrast, AIDS patients may develop chronic diarrhea, which can be fatal. Effective antiretroviral therapy can lead to resolution of AIDS-associated cryptosporidiosis, presumably due to improvement in the intestinal immune response. The long-term goals of this project are to determine the immune mechanisms involved in the control of cryptosporidiosis in healthy adults and AIDS patients on effective antiretroviral treatment. We have demonstrated that sensitized, immunocompetent volunteers expressed interferon gamma (IFN γ) in response to *C. parvum* exposure and that IFN γ expression was associated with resistance to infection. By contrast, naive, symptomatic individuals initially expressed Interleukin 15 (IL-15), which was associated with control of oocyst excretion. Neither IL-15 nor IFN γ was detected in AIDS-associated chronic cryptosporidiosis, but expression of IL-15 and IFN γ was noted in biopsies obtained from patients responding to antiretroviral therapy. Preliminary studies demonstrated that IL-15 can activate lymphocytes to lyse infected epithelial cells. However, many questions remain. For example, what are the effector mechanisms used by IL-15 and IFN γ , in the control phase and how are these responses coordinated? Can Th1 cytokines in fact lead to resolution of cryptosporidiosis in AIDS patients in the absence of immune recovery? What is the sequence of responses in AIDS patients with immune recovery with effective anti-retroviral therapy. The specific aims of the current proposal are: 1) To test the hypothesis that IL-15 and IFN γ help clear infection of epithelial cells by activation of cytolytic cells and establish the mechanisms used by the effector cells. 2) To confirm the importance of Th1 cytokines in resolution of cryptosporidiosis by conducting a pilot, proof-of-concept, open-label trial of IL-12 therapy in chronic cryptosporidiosis in AIDS patients not responding to antiretroviral therapy. 3) To confirm that mechanisms used by cytolytic cells defined in aim 1 and associated cytokines, effector molecules, and chemokines are expressed in the intestines in human cryptosporidiosis using microarray analysis of intestinal biopsies obtained before and after experimental challenge of immunocompetent adults with *C. parvum* oocysts. 4) To test the hypothesis that AIDS patients with cryptosporidiosis sequentially expresses innate and then Th1 memory responses during immune reconstitution. These studies should identify key aspects of the human immune response needed for vaccines to prevent cryptosporidiosis and identify the host responses that can be targeted for adjunctive immunotherapy for cryptosporidiosis in patients with AIDS and other immunodeficiencies. The results should also provide insights into the mechanisms involved in mucosal immunity to other intracellular pathogens.

Grant: 2R01AI041816-07
Program Director: LANG, DENNIS R
Principal Investigator: FREITAG, NANCY E PHD
Title: Listeria virulence gene expression within host cells
Institution: SEATTLE BIOMEDICAL RESEARCH SEATTLE, WA
INSTITUTE
Project Period: 1997/02/01-2006/03/31

DESCRIPTION (provided by the applicant): *Listeria monocytogenes* is a facultative, intracytoplasmic bacterial pathogen that is responsible for serious infections in immunocompromised patients, pregnant women, and neonates. *L. monocytogenes* infections are primarily food-borne and listeriosis is the leading cause of death from food-related illness. In addition to its significance as a human pathogen, *L. monocytogenes* also serves as a useful model system for exploring the intracellular interactions that take place between parasite and host. *L. monocytogenes* is capable of sensing the different host cell compartment environments it encounters during the course of infection and of responding with the regulated expression of virulence factors. The PrfA protein of *L. monocytogenes* is a key transcriptional activator of virulence gene expression. PrfA contributes to the temporal regulation of *L. monocytogenes* gene expression within host cells, but the mechanisms used by the bacterium to coordinate intracellular gene expression are unknown. The goal of these studies is to elucidate the mechanisms that govern intracellular gene expression and to identify *L. monocytogenes* gene products that are subject to this intracellular regulatory network. In Aim 1, experiments are designed to analyze the mutations in *L. monocytogenes* regulatory mutants with altered patterns of intracellular gene expression and to define the effects of these mutations on virulence gene regulation. These studies should lead to the identification of new components that may act in concert with PrfA and that contribute to intracellular regulation of gene expression. Aim 2 will define functional regions of PrfA that promote activation of virulence gene expression. The contributions of specific functional domains of PrfA to virulence gene regulation in *L. monocytogenes* will be assessed. In Aim 3, studies are designed to identify additional *L. monocytogenes* gene products whose expression or activity is PrfA-dependent, and assess the contributions of these products to bacterial pathogenesis. The proposed studies should further our understanding as to how an intracellular bacterium senses the environment of different host cell compartments and regulates expression and activity of its virulence factors in response. This information is important towards better definition of the interactions that occur between host and pathogen during the process of infection.

Grant: 2R01AI042490-04
Program Director: RUBIN, FRAN A.
Principal Investigator: GRAVETT, MICHAEL G
Title: Experimental Model for Chorioamnionitis and Prematurity
Institution: OREGON HEALTH & SCIENCE UNIVERSITY BEAVERTON, OR
Project Period: 1997/09/01-2006/05/31

Prematurity is the leading cause of neonatal morbidity and mortality in the United States. Intrauterine infections are an important, and potentially treatable cause of prematurity, and are associated with increased risk of neonatal white matter lesions of the brain and cerebral palsy. However, the mechanisms by which infection leads to prematurity and/or cerebral palsy remain speculative and treatment strategies untested largely because humans cannot be longitudinally studied following infection. We propose to use chronically instrumented pregnant rhesus monkeys at 120-130 day gestation with experimental intrauterine infection, as previously described (Gravett et al, Am J Obstet and Gynecol; 171:1660-1667,1994) to study the temporal and quantitative relationships among infection, cytokines, prostaglandins, steroid hormones, cytokine antagonists, preterm labor, and neonatal white matter lesions of the brain in order to develop effective interventional strategies. After postoperative stabilization in a tether, we will; (1) inoculate Group B Streptococci (GBS) into the amniotic fluid to establish intrauterine infection and preterm labor. Uterine contractility will be continuously monitored and periodic samples of amniotic fluid and maternal and fetal blood (1-4 cc) will be obtained for assays of eicosanoids, steroid hormones, cytokines, matrix metalloproteinases and for microbial studies; (2) utilize antibiotics with and without potent inhibitors of proinflammatory cytokine production (dexamethasone, IL-10) or prostaglandin production (indomethacin) to ascertain the most effective intervention to down-regulate the cytokine/prostaglandin cascade and associated uterine activity; (3) infuse proinflammatory cytokine IL-1beta into the amniotic cavity through indwelling catheters in the absence of infection. Prior to infusion of IL-1beta in the absence of infection, specific novel proinflammatory cytokine inhibitors (IL-1ra and sTNF-R1 PEG) will be used to identify other potentially useful immunomodulators. Samples of the decidua, fetal membranes, tissues, and brain will be obtained at cesarean section for microbiologic, histopathologic studies, immunohistochemistry for cytokines, localization and quantitation of mRNA for cytokines and PGHS-2. Fetal brain will be examined for increased apoptosis associated with white matter lesions. Leukocytes in amniotic fluid and tracheal aspirates will be assessed by flow cytometry. Postpartum, the mother will be treated with appropriate antibiotics to eradicate the GBS from the genital tract and returned to the colony. These studies will clarify the pathophysiology of infection-associated preterm labor and will suggest effective interventional strategies.

Grant: 1R01AI044374-01A2
Program Director: KLEIN, DAVID L
Principal Investigator: PIROFSKI, LIISE-ANNE MD
Title: VARIABLE GENE DEFECTS AND PNEUMOCOCCAL SUSCEPTIBILITY
Institution: YESHIVA UNIVERSITY NEW YORK, NY
Project Period: 2001/04/01-2006/03/31

DESCRIPTION (Adapted from Applicant's Abstract): The rationale for vaccination with pneumococcal capsular polysaccharide (PPS) vaccines is to induce opsonic antibodies to PPS, which are required for protection against *Streptococcus pneumoniae*. However, available PPS-based vaccines are poorly immunogenic in many patients at the highest risk for pneumococcal infection. Based on the following evidence we hypothesize that reduced expression of immunoglobulin genes from the VH3 subgroup translates into an impaired anti-PPS response: i) antibodies to PPS are oligoclonal and use genes from the VH3 subgroup; ii) reduced VH3 expression has been reported in patients at risk for pneumococcal infection that generate poor responses to PPS vaccines. This application proposes to determine structure-function relationships for human antibodies to serotype 3 *S. pneumoniae*, a cause of invasive pneumococcal infection in adults and children. The specific aims are: 1) To determine the molecular structure of human mAbs to PPS 3 generated in transgenic mice reconstituted with human immunoglobulin loci; 2) To characterize the infection in mice and as opsonic or non-opsonic in vitro; 3) To use mAbs with molecular structure as defined in Aim 1 and functional efficacy as defined in Aim 2 to select peptide mimics of PPS 3 epitopes, and to use the peptides to determine if PPS-elicited antibodies in patients recognize protective, non-protective or disease-enhancing epitopes. Our studies will provide evidence to support or refute the hypothesis that pneumococcal vaccine failure results from an inability to produce antibodies to PPS epitopes with a certain molecular structure. This work will provide a new scientific knowledge base regarding structure-function relationships for antibodies to PPS 3 that can be used clinically to evaluate vaccine efficacy and to develop more immunogenic vaccines for patients at risk for pneumococcal infection.

Grant: 1R01AI045533-01A2
Program Director: BAKER, PHILLIP J.
Principal Investigator: WINKLER, HERBERT H PHD
Title: Genomic Level Expression Patterns in Typhus Rickettsia
Institution: UNIVERSITY OF SOUTH ALABAMA MOBILE, AL
Project Period: 2001/03/01-2006/02/28

DESCRIPTION (provided by the applicant): This proposal is focused on the characterization of the global expression patterns of the *Rickettsia typhus* group *rickettsiae*. Members of the genus *Rickettsia* including *R. prowazekii*, the etiologic agents of epidemic typhus, are obligate intracellular bacterial parasites. We seek to understand the mechanisms by which *rickettsiae* cope with the problems and exploit the opportunities of the host cell cytoplasm, their unique and only niche. This is a niche that changes as the infection proceeds and as the host alternates from insect to mammal. *R. prowazekii* has only 834 genes. Our investigation will establish the gene-sets within these 834 genes that are expressed under changing environmental conditions by both members of the typhus group. The patterns of constitutive gene expression and regulated gene expression in response to environmental factors that are significant to the biology of *rickettsiae* will be determined. By coupling the recently completed genomic sequence of *R. prowazekii* and the advances in DNA Array technology with our expertise in transcriptional regulation and *rickettsial* biology, we can now characterize genetic expression in these obligate intracellular parasites in a global and comprehensive manner. Aim 1. Form a DNA Array on nylon filters containing all 834 genes of *R. prowazekii* Aim 2. Optimize the methods for synthesizing probes that will hybridize to the *rickettsial* genomic Array. Aim 3. Characterize the patterns of *rickettsial* genetic expression on a global and comprehensive scale. The total RNA will be extracted from infected host cells under conditions chosen to best demonstrate the regulatory diversity and adaptive biology of *rickettsiae*. The labeled-probes formed from all the *rickettsial* mRNAs present within a particular total RNA sample will be hybridized to the genomic Array of all *R. prowazekii* genes and analyzed to establish the complete repertoire of genes expressed under these conditions.

Grant: 1R01AI045537-01A2
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: FRIEDMAN, RICHARD L
Title: M. tuberculosis survival in Macrophages
Institution: UNIVERSITY OF ARIZONA TUCSON, AZ
Project Period: 2001/04/01-2004/03/31

DESCRIPTION (provided by the applicant): Central to the disease process in tuberculosis are the interactions of the bacilli with host macrophages. The infection of macrophages by *Mycobacterium tuberculosis* can be divided into four steps: adherence, entry, intracellular survival, and multiplication. This proposal will concentrate on one of these steps, survival. The role virulence factors of *M. tuberculosis* play in these complex interactions is virtually unknown. The aim of the proposed research is to identify, clone, and characterize genes and their protein products of *M. tuberculosis* which are required for intracellular survival within macrophages. Potential virulence factor genes of *M. tuberculosis* will be cloned by first constructing a recombinant library and incorporating it into the non-pathogenic *Mycobacterium smegmatis*. This rapidly growing mycobacterium is internalized and killed by macrophages. Clones with enhanced survival in macrophages will be identified and examined for the presence of *M. tuberculosis* genes involved in survival. Using this system we have already isolated a *M. tuberculosis* gene, named *eis* (enhanced intracellular survival gene), which does enhance intracellular survival of *M. smegmatis* within macrophages. The primary focus of this grant application is the further characterization of the *eis* gene and its protein product Eis. The specific aims are: 1. Effect of *eis* Gene Inactivation on Survival and Multiplication of *M. tuberculosis* in Macrophages and Mice. Mutations will be constructed in *eis* and introduced into the chromosome of both avirulent and virulent *M. tuberculosis* (H37Ra and H37Rv) by allelic exchange. The ability of the *eis* knockout mutants to survive and replicate in the U-937 macrophage survival assay and in human mononuclear phagocytes will be tested and compared to the parental strain. Additionally, the ability of the *eis* mutants to persist and replicate in vivo in a mouse intravenous infection model will also be evaluated. 2. Mechanism(s) Whereby *eis* Enhances Survival and Multiplication of *M. tuberculosis* in Unactivated and Interferon-gamma-Activated Macrophages. To learn how *eis* may enhance intracellular survival of mycobacteria in macrophages, survival in interferon-gamma-activated U-937 cells and human monocytes will be evaluated. The ability of *M. smegmatis* with and without *eis*, as well as wild-type *M. tuberculosis* and *eis* knock-out mutants, constructed in Specific Aim No.1, to survive/multiply in both unactivated and interferon-gamma-activated U-937 cells and human monocytes will be determined. Studies will also be done to determine what role Eis may play in the ability of *M. tuberculosis* to resist known killing mechanisms operating in macrophages. 3. Properties of the Eis Protein and their Relationship to its Survival-Increasing Action. These studies will include: (1) intracellular localization of Eis in *M. tuberculosis* and *M. smegmatis*, (2) purification of the Eis protein, (3) screening of sera from tuberculosis patients for presence of antibody to Eis, and (4) measurement of *eis* gene expression in vitro and within macrophages using integrative reporter gene vectors. 4. Identification of Non-*eis* Survival Genes in a New *M. tuberculosis* DNA Library. In initial studies, *eis*-containing clones were the predominate clones isolated after the sixth passage in the U-937 macrophage survival assay. Such *eis*-containing clones are preferentially selected and appear to out-compete/dominate other *M.*

tuberculosis genes which may also play a role in intracellular survival. Thus, in order to identify these other potential genes, a new *M. tuberculosis* plasmid library will be constructed with larger (10-12 kb) DNA inserts of genomic DNA from an H37Rv *eis* knockout mutant. This *eis* knockout library will then be screened for survival in the U-937 macrophage survival assay. Clones with enhanced survival will be isolated and further characterized.

Grant: 1R01AI045626-01A2
Program Director: MILLER, MARISSA A.
Principal Investigator: RICE, LOUIS B MD MEDICINE
Title: REGULATION OF AMPICILLIN RESISTANCE IN E. faecium
Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH
Project Period: 2001/03/01-2005/02/28

DESCRIPTION (Verbatim from Applicant's Abstract): The dramatic rise in prevalence of multi-resistant enterococci in United States hospitals over the past decade has limited therapeutic options, affected morbidity and mortality and increased the cost of caring for seriously ill hospitalized patients. The expression of resistance to vancomycin has received the most attention during this time. However, it is equally problematic that virtually all vancomycin-resistant enterococci (VRE) are *Enterococcus faecium* that express resistance to high levels of ampicillin. While it is clear that ampicillin resistance in *E. faecium* requires expression of low affinity penicillin-binding protein 5 (PBP5), the correlation between the amounts of detectable PBP5 and the level of ampicillin resistance is not exact. Several point mutations in *pbp5* have been identified in strains expressing high-level ampicillin resistance, but the specific contributions of these mutations to the levels of resistance have never been assessed. We have identified the first transferable ampicillin resistance described from *E. faecium* in a VRE strain from Northeast Ohio. The *pbp5* gene conferring resistance in this isolate possesses several mutations that have been associated with high-level ampicillin resistance in other *E. faecium* isolates. Curiously, levels of ampicillin resistance expressed by transconjugant *E. faecium* strains are not equivalent to those expressed by the donor, despite documentation that equivalent amounts of PBP5 are produced. In the past two years, we have acquired evidence that levels of ampicillin resistance expressed correlate with transcription (but not necessarily translation) of an upstream open reading frame designated *ftsW_{Ef}*. The specific aims of this proposal are to: 1) perform site directed mutagenesis of *E. faecium pbp5* to determine the functional (MIC, affinity) and structural importance of specific mutations. With collaborations in France and Switzerland, we now possess the molecular expertise to create the mutants and analyze their functional impact and determine the crystal structure; 2) to investigate the role of the putative upstream repressor *psr* in regulating expression of ampicillin resistance in *E. faecium*; 3) to investigate the mechanisms by which transcription of *ftsW_{Ef}* impacts the levels of ampicillin resistance expressed by *E. faecium*; 4) to assess whether upstream open reading frames designated *nanE-Ef* and *ywrF-Ef* affect levels of ampicillin resistance expressed and 5) to determine whether the peptidoglycan precursors differ in sensitive and resistant strains. These investigations will yield new insights into what is arguably the most resistant nosocomial pathogen of our time by providing important structure-function correlations for PBP5, correlations which may be important for the development of newer and better inhibitory compounds. They will also yield important new information on mechanisms of cell wall synthesis in *E. faecium* and other Gram-positive bacteria as well as on the mechanisms by which ampicillin resistance in *E. faecium* is regulated.

Grant: 1R01AI045673-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: LAFUSE, WILLIAM P PHD
Title: INHIBITION OF IFN GAMMA SIGNALING BY MYCOBACTERIA
Institution: OHIO STATE UNIVERSITY COLUMBUS, OH
Project Period: 2001/02/15-2005/01/31

DESCRIPTION (Adapted from the Applicant's Abstract): *M. tuberculosis* infects one third of the world's population. *M. avium*, a ubiquitous organism in the environment, is a major pathogen in AIDS patients. The interaction between mycobacteria and the macrophage is the critical step in the establishment of the infection. Mycobacteria are taken up by macrophages through phagocytosis and reside within phagosomes, where the bacteria survive and replicate. The principal defense mechanism of the macrophage against the mycobacteria is activation of the macrophage by IFN-gamma, resulting in increased mycobacterial killing. A more complete understanding of the resistance of the mycobacteria should help in developing new strategies for treatment and prevention of mycobacterial infections. Experimental studies in mice have shown a wide variation in virulence of different *M. avium* strains and the molecular basis for this difference in virulence as well as the relationship between virulence and the immune system is unknown. One possible mechanism is that virulent mycobacteria inhibit the ability of the infected macrophage to respond to IFN-g. Studies from the PI's laboratory have found that infection of mouse macrophages with a virulent strain of *M. avium* results in a dramatic reduction in gene expression induced by IFN-gamma. Analysis of the IFN-gamma signaling pathway, the JAK/STAT pathway, showed that mycobacterial infection inhibits IFN-gamma induced STAT1 activation and tyrosine phosphorylation. *M. avium* also inhibited tyrosine phosphorylation of JAK1, JAK2 and the IFN-gR alpha chain. This decrease in ability of the infected macrophages to respond to IFN-g was shown to coincide with a decrease in IFN-gamma receptor alpha and beta chain protein and mRNA expression. The PI has also observed that *M. avium* infection induces expression of SOCS-3, which has been shown to inhibit IFN-g signaling. These observations are the basis for the present application. The specific aims of the proposal are: 1) To determine the role of inhibition of IFN-g signaling in the virulence of *M. tuberculosis* and *M. avium*. 2) To determine the mechanism of IFN-gR inhibition in *M. tuberculosis* and *M. avium* infected macrophages. 3) To determine if receptor internalization by endocytosis and proteasomal/lysosomal degradation is responsible for the loss of IFN-g receptor in *M. avium* infected macrophages. 4) To determine the role of SOCS-3 in the inhibition of IFN-g signaling in *M. avium* infected macrophages.

Grant: 1R01AI045817-01A2
Program Director: AULTMAN, KATHRYN S.
Principal Investigator: FEDERICI, BRIAN A PHD
Title: Molecular Improvement of Bacterial Mosquito Larvicides
Institution: UNIVERSITY OF CALIFORNIA RIVERSIDE RIVERSIDE, CA
Project Period: 2001/07/01-2005/06/30

DESCRIPTION (provided by the applicant): The long-term objectives of the proposed research are to develop more effective and environmentally safe recombinant bacteria for controlling the mosquito vectors of major human diseases including malaria, filariasis, dengue, and the viral encephalitides. These bacteria will be significantly more cost-effective than *Bacillus thuringiensis* subsp *israelensis* (Bti) and *Bacillus sphaericus* (Bs), the two species currently used in operational vector control programs. In addition, they will be much less prone to induce mosquito resistance, which has already developed to *B. sphaericus* in field populations of *Culex* mosquitoes in Brazil, China, and India. To support the sustainable use of these new bacteria in vector control programs, novel combinations of insecticidal proteins will be used to evaluate efficacy and resistance management strategies aimed at controlling mosquito species belonging to the most important vector genera, namely, *Anopheles*, *Aedes*, and *Culex*. The development and use of these new recombinants will be enhanced by studies focusing on improving our knowledge of mechanisms underlying the synergism responsible for the high toxicity and capacity of the Cyt1A protein to delay, avoid, or overcome resistance in vector populations to other bacterial endotoxins. These objectives will be achieved through a comprehensive research program consisting of the following three specific aims: (1) Construction of improved bacterial insecticides based on novel combinations of mosquitocidal endotoxins; (2) Determination of the target spectrum and toxicity of the bacterial recombinants; and (3) Determination of the general mechanism by which Cyt1A synergizes endotoxins and overcomes resistance. Bacterial insecticides developed through this research should result in improved vector control and disease reduction, with concomitant health benefits accruing from reductions in the use of broad-spectrum synthetic chemical insecticides. Moreover, the insecticidal protein combinations identified to optimize efficacy and resistance management will provide models for engineering field populations of bacteria and algae for vector control, and for resistance management programs for Bt transgenic crops.

Grant: 1R01AI045836-01A2
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: 2001/06/01-2005/04/30

DESCRIPTION (Adapted from the Applicant's Abstract): This proposal is in response to the program announcement 'Research on molecular immunology of STDs (ROMIS).' Interferon (IFN)- γ 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 IFN γ , 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 DNase I footprinting, 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 to 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 an otherwise effective immunological response, and to understand regulation of this response in order to overcome Chlamydia's evasive mechanisms.

Grant: 1R01AI045871-01A2
Program Director: BAKER, PHILLIP J.
Principal Investigator: YU, XUE-JIE MD
Title: MOLECULAR BASIS OF PERSISTENT EHRLICHIA INFECTION
Institution: UNIVERSITY OF TEXAS MEDICAL BR GALVESTON, TX
GALVESTON
Project Period: 2001/02/01-2005/01/31

DESCRIPTION (Adapted from the Applicant's Abstract): Persistent infection has been observed for all ehrlichial species in a variety of animals and in humans. Understanding the mechanism of persistent ehrlichial infection will enable design of new therapeutic approaches to treat ehrlichial infections and vaccines to prevent the diseases. The following hypotheses are presented: 1) persistent infection is caused by antigenic variation and/or differential expression of the outer membrane protein (OMP) genes of Ehrlichia and 2) the OMPs confer immunity in the host against re-infection by the pathogen. The subject of this proposal is Ehrlichia chaffeensis which causes a newly emerging infectious disease, human monocytotropic ehrlichiosis. Persistent infection by E. chaffeensis has been documented in human and animals. A canine model will be employed to study the mechanism of this persistent infection. In specific aim 1, antigenic variation of E. chaffeensis gp120 will be analyzed in dogs infected by tick transmission. Canine blood will be obtained over a six month period, the gp120 gene will be amplified by PCR and sequenced and compared to the gene in the infecting isolate to determine the possible deletion or insertion of repeats in the gp120 gene. In addition, changes in the size and antigenicity of gp120 reisolated from infected canine blood will be examined. Specific aim 2 will entail analysis of the differential expression of the p28 multigene family. This will be accomplished by RT-PCR or epitope mapping. Aim 3 will involve testing of the protective immunity elicited in canines by immunization with recombinant OMPs, followed by challenge of immunized animals with E. chaffeensis. This will provide valuable information regarding the potential use of these antigens as subunit vaccines.

Grant: 1R01AI046445-01A1
Program Director: MILLER, MARISSA A.
Principal Investigator: JI, GUANGYONG PHD
Title: CONTROL OF STAPHYLOCOCCAL VIRULENCE BY QUORUM SENSING
Institution: HENRY M. JACKSON FDN FOR THE ADV ROCKVILLE, MD
MIL/MED
Project Period: 2000/12/08-2005/11/30

DESCRIPTION (Verbatim from Applicant's Abstract): The goal of this project is to determine the mechanism of peptide-determined autoinduction of virulence gene expression in *Staphylococcus aureus* with the long-term objective of defining how this regulation contributes to pathogenesis of *S. aureus* diseases. The accessory gene regulator (agr) regulon is the central regulatory system that controls the expression of virulence genes in *S. aureus*. This agr regulon includes: 1) a two-component signal transduction system (AgrA/AgrC); 2) a modified peptide (autoinducing peptide or AIP) that is probably secreted via AgrB, processed from the AgrD propeptide, and used as the ligand for the AgrC sensor; and 3) an RNA molecule, RNAIII, that is the actual regulator of the agr response. We discovered that AIPs produced by some *S. aureus* strains inhibited the agr response in other strains, and we identified three groups of *S. aureus* strains that exhibited distinct activation-inhibition activities. Sequence analysis of the agr loci for selected members of each of these three groups revealed a striking pattern of group-specific sequence conservation and divergence. We propose in this application to delineate the steps required for AIP production. We hypothesize that the processing of AgrD propeptide and the secretion of the AIP are jointly catalyzed by AgrB. To test these proposals, we will pursue two specific aims designated to: (1) analyze the mechanisms of AgrD processing and AIP secretion, and (2) determine the function of AgrB in AgrD processing and AIP secretion and identify the catalytic domains and the critical amino acid residues involved in these processes. Genetic and biochemical methods will be used for both aims. The results obtained will facilitate our understanding of this complex agr system and may result in the development of new therapeutic antistaphylococcal drugs that target agr.

Grant: 1R01AI046534-01A2
Program Director: KORPELA, JUKKA K.
Principal Investigator: KNIGHT, PAUL R MD
MULTIDISCIPLINARY: MULTIDISCIPLINARY
LIN, BASIC MED
Title: Host response to post-operative pneumonia
Institution: STATE UNIVERSITY OF NEW YORK AT AMHERST, NY
BUFFALO
Project Period: 2001/07/01-2006/05/31

DESCRIPTION (Verbatim from the Applicant's Abstract): Anesthesia/surgery predisposes the patient to develop nosocomial pneumonia by mechanisms that are not completely defined. The presence of a viral respiratory tract infection (RTI) during anesthesia/surgery further complicates the host antibacterial response. Evidence from our laboratory has demonstrated anesthesia/surgery induces changes in cytokine response (e.g., TNFalpha, MIP-2, IFNgamma), leukocyte recruitment, and lung injury to influenza RTI. These responses are also critical to innate host defenses against bacterial pathogens. Our focus is to examine cellular mechanisms during a viral RTI that predispose the host to a post-surgical bacterial pneumonia. We hypothesize that anesthesia/surgery will change host responses differently during distinct periods in the course of a viral RTI by altering expression of pro- and antiinflammatory cytokines, thereby decreasing antibacterial defenses. Aim #1 will assess the effects of anesthesia/surgery during influenza on bacterial clearance, inflammatory cell influx, and cytokine expression on Escherichia coli challenge. We predict that laparotomy during influenza will promote the relative expression of MCP-1 and IL-10 over TNFalpha, MIP-2, and IFNgamma. Aim #2: will assess ex vivo the combined effect of laparotomy and influenza on a) LPS stimulated aMphi cytokine expression and phagocytic activity, and b) the ability of in vitro antiMCP-1, antiIL-10, or IFNgamma administration to improve M dysfunction. We postulate that laparotomy during influenza will alter aMphi regulatory functions and decrease effector functions as a result of selective enhancement of expression anti-compared to proinflammatory cytokines. Finally, in Aim #3, we will examine the contribution of endogenous cytokines in the suppression of antibacterial defenses following laparotomy during influenza by selective cytokine manipulations. Bacterial clearance, inflammatory cell influx, and cytokine levels will be assessed. We anticipate that neutralization of IL-10 or MCP-1, administration of IFNgamma, or increased TNFalphaexpression will improve antibacterial host defenses following laparotomy during physical signs of influenza. These studies will examine mechanisms that lead to alterations in bacterial clearance post-surgically following a viral RTI, assess the pathogenesis of post-surgical pneumonia in general, and suggest immune adjuvant strategies to prevent this complication.

Grant: 1R01AI046589-01A2
Program Director: LANG, DENNIS R
Principal Investigator: MILLER, VIRGINIA L
Title: Virulence Networks in Salmonella
Institution: WASHINGTON UNIVERSITY ST. LOUIS, MO
Project Period: 2001/04/01-2006/02/28

DESCRIPTION (Applicant's Abstract): Salmonella is one of the most extensively characterized bacterial pathogens and is a leading cause of bacterial gastroenteritis. With the aging of the population we are likely to see further increases in the incidence of this infection, and we can expect an increase in the severity of disease because the elderly are more susceptible to disease and tend to have more severe infections. It is clear that the SPI1 encoded type III secretion apparatus and the proteins secreted by this apparatus play a major role for invasion of the epithelium, recruitment of PMNs, and diarrhea. A better understanding of this system and its role in pathogenesis of diseases caused by Salmonella should lead to better methods of treatment and prevention. We recently identified the sigDE locus of *S. typhimurium*; sigD encodes an effector protein secreted by the SPI1 type III secretion apparatus (note: effector proteins are those believed to have a direct effect on host cells). Like other effectors in this system, expression of sigDE requires SirA and HilA, but as with other effectors, expression of sigDE is probably not directly affected by these regulators. The current model suggests that SirA turns on expression of HilA which turns on expression of invF. InvF is thought to activate expression of genes encoding the effectors; we have demonstrated that InvF is required for sigD expression. In addition, a screen for regulators of sigD identified SicA (encoded by SPI1). SicA is thought to be a chaperone for SipB and SipC. How expression of the type III secretion system and expression of the translocators and effector proteins is coordinated remains an important question. These are the questions we hope to address in this proposal with the long-term goal of understanding how these events are integrated with other virulence factors of Salmonella to cause disease. Specifically we propose the following: Aim 1. Which transcriptional units are regulated by InvF and SicA? To test this we will identify the key transcriptional units and will examine expression of reporter constructs in different mutant backgrounds. Aim 2. How do SicA and InvF mediate regulation of the sigDE and other promoters? To do this we will examine the interactions of SicA and InvF with each other and the promoters they regulate. Aim 3. To identify other effectors secreted by the SPI1 type III secretion pathway by identifying InvF regulated genes. These will be characterized for their role in virulence related assays.

Grant: 1R01AI046706-01A2
Program Director: MILLER, MARISSA A.
Principal Investigator: PIER, GERALD B PHD MICROBIOLOGY, OTTUMWA
Title: Virulence and Immunity to Staphylococci
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 2001/02/01-2006/01/31

DESCRIPTION (Verbatim from Applicant's Abstract): The long-term goal of this study is to understand the role in pathogenesis and immunity of an environmentally regulated surface polysaccharide of *Staphylococcus aureus* chemically characterized as poly-N-succinyl-B-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 representative of major lineages 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 several animal systems of *S. aureus* infection, including animals actively and passively immunized with *ica*-deleted *S. aureus* and normal human serum to reflect the immunologic status of humans, who have high levels of natural antibody to *S. aureus* surface antigens. 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. Rabbits will be immunized with the variants and sera assessed for antibody titer and opsonic killing ability. The PNSG variant structures will be used to immunize mice to evaluate their ability to generate protective immunity in the same systems used for the study of the role of PNSG in *S. aureus* virulence. In addition, passive protection by the rabbit sera raised to the variant PNSG constructs will be evaluated in the animal systems. All the above mentioned studies will provide new and useful information regarding pathogenesis and immunity of staphylococcal infections, stressing the use of animal systems that reflect naturally acquired immunity in humans to *S. aureus*. 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 systems, 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: 1R01AI047141-01A1
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: CORNELISSEN, CYNTHIA N PHD
Title: A MOLECULAR STUDY OF THE GONOCOCCAL TRANSFERRIN RECEPTOR
Institution: VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA
Project Period: 2001/01/01-2005/12/31

DESCRIPTION (Verbatim from Applicant's Abstract): Transferrin-binding proteins A and B (TbpA and TbpB) are components of a surface-exposed, human transferrin receptor that is expressed by *Neisseria gonorrhoeae*. Because these proteins are well conserved and not subject to phage or antigenic variation, they have been focused on as potential vaccine candidates. The overall goal of this proposal is to determine how all of the components of the gonococcal transferrin receptor complex work and if both characterized components are immunogenic. The specific aims of the proposal address the following questions: 1. How are all of the components of the transferrin-receptor complex organized in the gonococcal outer membrane to facilitate transferrin interaction and transferrin-iron acquisition? Determining the topology and stoichiometry of this receptor is critical for our understanding of what regions are required for function and what domains are accessible to the immune system. Deletion, insertion and site-specific mutagenesis will be performed on TbpA and TbpB to establish structure/function relationships in the characterized components of the receptor. 2. What is the molecular mechanism that coordinately regulates the expression of *tbpA* and *tbpB*? Identifying the promoter and other regulatory regions that effect *tbpA* and *tbpB* expression will lead to a better understanding of how optimal levels of Tbp proteins are achieved and maintained. 3. Do the components of the transferrin-receptor generate an immune response, and if so, what are the biological activities of Tbp-specific antibodies? Mucosal secretion and serum samples of *N. gonorrhoeae*-infected men and women will be screened for the presence and type of antibodies against the components of the transferrin receptor. With any antibodies we detect, we will conduct functional assays including transferrin blocking, complement-mediated killing and phagocytic killing assays. We will also immunize rabbits with all or parts of the transferrin receptor to determine whether functional antibodies can be elicited.

Grant: 1R01AI047181-01A2
Program Director: LANG, DENNIS R
Principal Investigator: BOST, KENNETH L
Title: Limited IL-12B2 receptor expression during salmonellosis
Institution: UNIVERSITY OF NORTH CAROLINA CHARLOTTE, NC
CHARLOTTE
Project Period: 2001/05/01-2004/04/30

DESCRIPTION (provided by applicant): A critical driving force for optimal development of T helper type 1 (TH1) lymphocytes is signaling through the IL-12 receptor. The IL-12 receptor is composed of two subunits, with expression of the IL-12 receptor beta 2 chain (IL-12RB2) dictating a high affinity IL-12 receptor complex. Signaling through this high affinity IL-12 receptor controls the development of TH1 lymphocytes and the maintenance of this phenotype, while limiting lineage commitment to the TH2 phenotype. Since TH1 lymphocytes mediate cellular immunity, while TH2 lymphocytes enhance humoral responses, early expression of the high affinity IL-12 receptor is critical for a commitment to cell mediated immune responses. Salmonella is an intracellular pathogen of macrophages, epithelial cells and possibly dendritic cells, and requires cell-mediated immunity for clearance. Based on recently published work, we demonstrated that Salmonella-infected macrophages can significantly limit IL-12RB2 expression on T lymphocytes early in the response. This finding has profound implications for the early development and commitment of T lymphocytes to the TH1 lineage during Salmonella infection. The overall goal of this proposal is to define the mechanisms for Salmonella-induced reductions in IL-12RB2 expression in vitro and in vivo. At present, it is not clear whether induced reductions in IL-12RB2 expression are solely mediated by soluble factors or require macrophage-T cell contact. IL-12RB2 expression will be quantified at the level of mRNA using quantitative RT-PCR, and at the protein level using Western blot, FACS and radioreceptor analyses. Furthermore, reductions in T lymphocyte function associated with the loss of IL-12RB2 will be assessed, and a functional assessment of developing TH1 and TH2 lymphocytes will be determined by following STAT-4 activation, and T-bet, GATA-3 and c-maf mRNA expression, respectively. Whether infected dendritic cells can induce such alterations in CD4+ T cells will also be determined. Taken together these studies represent the first to define mechanisms whereby an intracellular bacterial pathogen can adversely affect the early development of TH1 lymphocytes upon infection.

Grant: 1R01AI047197-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: BRENNAN, PATRICK J
Title: LEPROSY BACILLUS,: FROM GENOTYPE TO PHENOTYPE
Institution: COLORADO STATE UNIVERSITY-FORT FORT COLLINS, CO
COLLINS
Project Period: 2001/06/15-2006/04/30

DESCRIPTION (Adapted from the Applicant's Abstract): The purpose of this completely revamped resubmission is the identification and implementation of new antigens for the diagnosis and epidemiological monitoring of leprosy and is based on the just-sequenced contiguous *M. leprae* genome, on the investigators' singular access to the bacillus, and driven by the extraordinary epidemiological conundrum of a convergence of falling prevalence and rising incidence. The other hypothesis-driven thrust is that a defective genotype must be reflected in a truncated phenotype, the definition of which will also provide knowledge and tools to help explain the peculiarities of leprosy, such as obligate intracellularism, characteristic cell tropism and reactions. S. Cole (Institut Pasteur) will complete the analysis and annotation of the genome and generate databases to provide the means for a two-laboratory effort (L'Institut Pasteur; CSU) to define the transcriptome, polymorphism within the infectious agent, and the entire simplified proteome through cloning of targeted genes and definition of the full array of in vivo expressed proteins. CSU will apply the genome and the structural knowledge of mycobacteria to define the simplified secondary gene products (LepLAM; LM; new extracellular lipids; peptidoglycan; etc.). CSU, Fio-Cruz (C. Pessolani), and Yonsei University (S.-N. Cho) will combine to test new products in guinea pigs for DTH responses, against PBMC's of leprosy patients for appropriate T-cell reactivity, and against sera for antibody responses, with a view to advancing the new skin antigen initiative and providing new blood assays for early diagnosis and identification of at risk populations.

Grant: 1R01AI047202-01A2
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: KALTENBOECK, BERNHARD PHD
Title: Chlamydia pneumoniae vaccine candidates
Institution: AUBURN UNIVERSITY AT AUBURN AUBURN, AL
Project Period: 2001/09/01-2005/06/30

DESCRIPTION (Provided by Applicant): Chlamydia (C.) pneumoniae is a major agent of community-acquired upper and lower respiratory infection and pneumonia. Increasing evidence suggests that C. pneumoniae infection plays an integral role in atherosclerotic coronary heart disease in developed countries, making C. pneumoniae a major public health concern. This clearly merits an effort to develop a vaccine against C. pneumoniae for prevention or treatment of respiratory disease, and possibly coronary heart disease and atherosclerosis. Using our new genetic immunization technologies, we are able to deconvolute the genomes of pathogens into the best vaccine candidates, and have recently validated this in a mouse model of C. psittaci infection in which we found 10 protective genes that also protect the original host animal. Perusing the complete genome sequence of C. pneumoniae, we propose as first step towards a C. pneumoniae vaccine to i) examine the C. pneumoniae homologs of the protective C. psittaci genes for protective efficacy in a mouse model of C. pneumoniae respiratory infection; ii) conduct a C. pneumoniae genome-wide search for the best antigens mediating prophylactic immunity against respiratory infection; and iii) perform experiments to understand the mechanisms for the success of such immunological intervention. We propose the following specific aims: 1) Test the C. pneumoniae homologs of the 10 protective C. psittaci genes in a mouse prophylactic respiratory model of C. pneumoniae infection. 2) Screen all approximately 1,000 C. pneumoniae genes for their protective efficacy in the mouse prophylactic model. 3) To understand the spectrum of possible responses in an outbred human population, dissect the immunological mechanisms of disease protection mediated by the C. pneumoniae vaccine candidate proteins in respiratory disease models using several inbred mouse strains.

Grant: 1R01AI047219-01A2
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: BANERJEE, ARESH PHD
Title: ROLES OF PILIN GLYCAN IN PATHOGENESIS OF N. GONORRHOEAE
Institution: CATHOLIC UNIVERSITY OF AMERICA WASHINGTON, DC
Project Period: 2001/07/01-2006/05/31

Neisseria gonorrhoeae (gonococcus, GC) not only causes uncomplicated gonorrhea but also complications like pelvic inflammatory disease and disseminated gonococcal infection. Pilus is an important pathogenicity factor of GC and is a polymer of the glycoprotein pilin. Glycans of pilin were proposed to play important role in GC pathogenesis. We have cloned a pilin galactosyl transferase (pgtA), that adds an alpha galactosyl. In most complicated disease strains, pgtA contains a poly-G tract like the other phase-variable genes that are frequently turned on and off to presumably provide GC with selective advantage in different niches of human body. However, in uncomplicated gonorrheal isolates, this poly- G is generally not present in pgtA. Hence, we hypothesize that the poly-G mediated high-frequency phase-variation of pgtA plays an important role in conversion of uncomplicated gonorrhea to more complex systemic diseases. In addition, our High pH Anion Exchange Chromatographic analysis of GC pilin glycans suggests presence of sugars that were not reported before. These observations lead to the hypothesis that GC pilin glycans are considerably more complex than the current models and some of these glycans can be associated with certain disease phenotypes or with particular sites of the human body. To test the stated hypotheses, the following specific aims are proposed: 1) Characterization of GC pilin glycans from selective strains and pilus glycosylation mutants and identification of novel pilin glycosylation genes; and 2) Examination of the role of pilin glycans in GC pathogenesis. These studies will further elucidate the mechanism of gonococcal infections. Additionally, glycosylation of many surface proteins, including pilins, of several pathogenic bacteria is being reported. Therefore, the study of the role of pilin glycans in GC pathogenesis is likely to provide important leads into the mechanisms of pathogenesis of other bacteria as well.

Grant: 1R01AI047242-01A1
Program Director: LANG, DENNIS R
Principal Investigator: COOKSON, BRAD T BS
Title: CD4+ T CELLS RESPONDING TO SALMONELLA INFECTION
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2001/01/01-2005/12/31

DESCRIPTION (Adapted from the Applicant's Abstract): Salmonella, Mycobacterium and Histoplasma are facultative intracellular pathogens that live inside phagosomes of host macrophages. They all cause AIDS-defining illnesses, and the investigator's long-term goal is to understand the development of immunity against such pathogens. CD4+ T cells are also required for immune mice to resist virulent Salmonella, providing a model of protective host functions which can successfully combat a macrophage-tropic infection. However, the specific bacterial antigens (Ags) recognized by Salmonella-immune hosts are largely unknown. Two proteins expressed in the surface-exposed "compartment" of Salmonella are recognized by CD4+ T cells from immune mice. One is a flagellar protein also recognized by T cells from humans immunized with Salmonella. The other is an unidentified protein expressed by most Enterobacteriaceae, including E. coli, Yersinia, Shigella, and Enterobacter. Both proteins are regulated in a fashion suggesting part of the Salmonella intracellular survival strategy is to down-regulate expression of bacterial surface Ags recognized by CD4+ T cells. In AIM 1, the diversity of Salmonella Ags recognized by CD4+ T cells from immune mice will be determined using SDS-PAGE fractionated bacteria as Ag, and bacterial expression of these Ags will be characterized with respect to compartmentalization and regulation using T cell clones. The studies will provide insight into the nature of Ags recognized by CD4+ T cells, the environmental signals affecting bacterial processing Salmonella for T cell responses. In AIM 2, genes encoding Ags recognized by T cell clones will be identified by expression cloning or sequencing analysis of biochemically purified Ags. This work may reveal gene products useful as markers of cellular immunity to Salmonella in humans. In AIM 3, murine infection with Salmonella strains expressing a model Ag in various compartments of the bacterial cell will be used to directly test if compartmentalization of bacterial Ag alters its significance for surveillance by T cells. Primary and secondary CD4+ T cell responses generated by these strains will be quantified using ELISPOT and flow cytometry, and the effectiveness of an Ag-specific immune response against these strains will be tested in vivo. These studies will provide insight into the nature of Ags recognized by CD4+ T cells responding to pathogens similarly adapted for life in phagosomes. In AIM 4, the functional importance of Ags identified in AIMS 1 & 2 will be determined by testing purified Ags for their ability to stimulate protective immunity against challenge by virulent Salmonella. The protective Ags identified will be excellent candidates for components of subunit vaccines and markers of cellular immunity in humans.

Grant: 1R01AI047313-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: FURIE, MARTHA B PHD
Title: ENDOTHELIAL RECRUITMENT OF T CELLS IN LYME DISEASE
Institution: STATE UNIVERSITY NEW YORK STONY STONY BROOK, NY
BROOK
Project Period: 2000/12/15-2005/11/30

DESCRIPTION (Adapted from the Applicant's Abstract): This project proposes to investigate the interactions between T lymphocytes in the context of chronic inflammation and infection. The hypothesis is that *B. burgdorferi* infection activates the vascular endothelium, enhancing extravasation of T lymphocytes that secrete type 1 proinflammatory cytokines. To test this central hypothesis, the PI will use in vitro models to pursue the following specific aims: 1) Determine the subpopulations of T lymphocytes that migrate across endothelium exposed to *B. burgdorferi*; 2) Identify the adhesion molecules and chemo-attractants involved in the migration of T lymphocytes across spirochete-stimulated endothelium; and 3) Explore the capacity of the host cytokine, interleukin (IL)10, to modulate interactions of T lymphocytes with endothelium activated by *B. burgdorferi*. These studies will provide greater insight into how accumulation of T lymphocytes is regulated not only in Lyme disease, but also in chronic inflammation in general.

Grant: 1R01AI047341-01A1
Program Director: SAVARESE, BARBARA M.
Principal Investigator: CHACKO, MARIAM R MD
Title: STD Screening in Young Women: A Stage-based Intervention
Institution: BAYLOR COLLEGE OF MEDICINE HOUSTON, TX
Project Period: 2001/03/01-2004/02/28

DESCRIPTION (provided by applicant): The broad long-term objective of the proposed 3-year project is to decrease the duration of untreated gonococcal and chlamydial infection in urban adolescent and young adult women through promotion of STD screening. Using the Trans Theoretical Model of Change as a conceptual guide, the specific aim of this application is to use a randomized clinical trial design to determine the efficacy of a client-centered, stage-based intervention promoting STD screening in response to high-risk sexual behavior in young sexually active minority women ages 16 to 22 years at an urban reproductive health clinic. In Phase 1, the intervention will be refined and adapted to the clinic setting. Information from preliminary studies (to date and in progress) will be used to complete this process. Two assessment focus groups will be conducted to review the completed intervention components. The components will be packaged into a final version and the health educators trained in motivational counseling. In Phase 2, 430 adolescent and young adult women, 16 to 22 years seeking reproductive health care will be recruited by a research assistant from a single clinic site for a 12-month project. Subjects will then be randomized to one of two conditions: the intervention + standard care (intervention group) or a standard care alone control (control group). Subjects in the intervention group will receive stage-based counseling by a Health Educator at the baseline, 2-week, and 6-month visits. The objective of the intervention is to promote STD screening in response to high-risk sexual behavior. Subjects in the intervention and control groups will receive standard care counseling from clinic staff at baseline, and scheduled 6-month visits and at unscheduled visits between the baseline and 12-month visits. To evaluate the efficacy of the intervention, subjects from both groups will be asked to complete face-to-face assessments at the baseline, 6- and 12-month visits. At all scheduled and unscheduled visits, subjects in both groups will be tested for gonorrhea and chlamydia infection by urine BDProbeTec. The primary outcome measure will be the number of clinic visits in response to high-risk sexual behaviors (scheduled and unscheduled) over 12 months. Secondary outcomes will include progression through the Stages of Change for STH screening, the frequency of consistent condom use during the past 30 days and number of episodes of gonococcal or chlamydial infections over 12 months.

Grant: 1R01AI047400-01A1
Program Director: LANG, DENNIS R
Principal Investigator: HOLLAND, MICHAEL J PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: IDENTIFICATION OF HELICOBACTER PYLORI VIRULENCE GENES
Institution: UNIVERSITY OF CALIFORNIA DAVIS DAVIS, CA
Project Period: 2001/02/01-2005/01/31

DESCRIPTION (Adapted from the Applicant's Abstract): *H. pylori* chronically infects gastric mucosa and is implicated in the pathogenesis of human gastritis, peptic ulcer disease, and gastric cancer. This proposal outlines a functional genomics-based approach designed to identify and characterize *H. pylori* virulence genes associated with human gastric disease. The proposed experiments will provide a functional map of the *H. pylori* genome which complements the complete sequences of two fully sequenced *H. pylori* genomes. A method termed "kinetically monitored reverse transcriptase-initiated PCR"; (kRT-PCR) forms the basis for the experimental plan. This method was developed in the laboratory of the principal investigator, and permits high throughput transcript quantitation. Genome-wide transcript profiling is proposed for *H. pylori* in certain benchmark states. The effects of growth phase, pH shift, iron, and human epithelial cells on *H. pylori* gene expression will be analyzed. These growth conditions, as well as knockout mutations within the *cag* pathogenicity island and within specific transcription factor genes, will be tested for their effects on the *H. pylori* transcriptome. Candidate virulence genes identified from the genome sequence include those involved in molecular mimicry, LPS biosynthesis, outer membrane proteins, candidate "phase variation" genes, and several hundred *H. pylori*-specific genes identified by comparisons of *H. pylori* genome sequences with those of other pathogenic and non-pathogenic bacteria. The *H. pylori* transcriptome will be organized into operons. Operon structures and coordinate expression profiles should reveal potential functions for unknown ORFs. Gene expression profiles for *H. pylori* in vivo will be obtained using a non-human primate model. Transcript profiles obtained under a variety of in vitro conditions will be organized into relational data sets and analyzed to reveal transcriptional paths and networks operative in *H. pylori*.

Grant: 1R01AI047759-01A2
Program Director: LAMBROS, CHRIS
Principal Investigator: GANGJEE, ALEEM PHD MEDICINAL CHEMIST
Title: Third Generation Antiopportunistic Agents
Institution: DUQUESNE UNIVERSITY PITTSBURGH, PA
Project Period: 2001/09/30-2004/08/31

DESCRIPTION (Provided by the applicant): The object of this proposal is the synthesis of a third generation of compounds identified via two iterative processes of synthesis, biological evaluation and X-ray crystal structure determinations as potent and selective inhibitors of dihydrofolate reductase (DHFR) from *P. carinii* (pc), *T. gondii* (tg) (and *M. avium* (ma)) and as potential clinical candidates for the treatment of infections caused by these organisms in patients with AIDS. The analogues in this study were designed on the X-ray crystal structures of previous first and second generation compounds with pcDHFR. The synthesis of the analogues is proposed via modifications of procedures from our laboratory and by established literature methods. The analogues will be evaluated as inhibitors of pcDHFR, tgDHFR and maDHFR with rat liver (rl) and human DHFR as reference enzymes. Initially, 16 analogues of the 36 proposed analogues will be synthesized and evaluated. The selectivity and potency will determine if other analogues in each Series I-IV will be synthesized or not synthesized. Thus synthetic targets will be determined by the biological activity. Potent and/or selective analogues will be further evaluated against *P. carinii*, *T. gondii*, *M. avium* and human foreskin fibroblast cells in culture (for cytotoxicity) and subsequently in mouse infection models of *P. carinii* and *T. gondii*. The biological evaluations will be carried out on a collaborative basis. If a problem is encountered with any of the analogues with respect to solubility, a reformulation of the analogue in different salt forms will be prepared to optimize in vivo activity as a prelude to clinical trials. Preformulation and formulation studies to afford the most appropriate route of administration and dose are proposed which includes in vitro and in vivo biopharmaceutic and pharmacokinetic characterization of selected analogues. For the first year two analogues previously identified as active in in vivo studies, will be developed to further improve effectiveness of these analogues in vivo. X-ray crystal structures of selected analogues with DHFRs will also be determined collaboratively. This study will provide third generation anti-opportunistic agents with selectivity and potency against pcDHFR, tgDHFR and maDHFR and should provide selective, less toxic, clinically useful agents, which overcome the drawbacks of currently, used regimens. Screening against tuberculosis (NIAID) and tumor cells in culture (NCI) is also proposed.

Grant: 1R01AI047841-01A1
Program Director: RUBIN, FRAN A.
Principal Investigator: KITTEN, TODD O BA
Title: Streptococcal virulence factors in extra-oral disease
Institution: VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA
Project Period: 2001/07/01-2004/05/31

Infective endocarditis affects about 22,000 people per year in the U.S. and kills over 2,000. This disease is caused primarily by viridans streptococci, and among viridans streptococci, primarily by *Streptococcus sanguis*. Previous studies concerning the virulence of streptococci for endocarditis have centered upon the examination of suspected virulence factors. The proposed research takes an entirely different approach, that of directly identifying new virulence factors. This will be done by adapting the technique of signature-tagged mutagenesis (STM) for use with *S. sanguis*. This approach requires no prior assumptions concerning the importance of any particular gene or activity in disease causation. Instead, it relies on the infection process to identify the bacterial genes needed for disease and their relative contributions. An *S. sanguis* strain that is also the subject of a separate ongoing genome sequencing project will be randomly mutagenized by STM using an in vitro transposition approach. The resulting mutants will be pooled and inoculated into rats serving as models for endocarditis. The STM approach will allow for the identification of mutants within the pool possessing increased or decreased virulence. The apparent altered virulence in these mutants will be verified by in vivo competition assays. Further characterization of mutants will include DNA sequence analysis to identify the mutated genes, further genetic manipulation to verify the contribution of the mutated genes, in vivo infectivity assays, and in vitro functional assays. It is anticipated that the characterization of mutants with altered virulence will result in a better understanding of the pathogenesis of endocarditis and will identify promising novel targets for vaccine or therapeutic development.

Grant: 1R01AI047852-01A1
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: RAMPHAL, REUBEN
Title: FLAGELLIN GLYCOSYLATION AND VIRULENCE OF P. AERUGINOSA
Institution: UNIVERSITY OF FLORIDA GAINESVILLE, FL
Project Period: 2001/07/01-2006/05/31

Post translational modification of proteins by covalent attachment of carbohydrate moieties is common among eukaryotes, but by comparison, relatively rare in bacteria. Currently, two virulence factors of *P. aeruginosa*, pili and the a-type flagellin as well as a protein responsible for phospholipase transport, are reported to be glycosylated. The long term goal of this application is therefore to understand the role of glycosylation in virulence of *P. aeruginosa*. The specific aims of this application are intended to study the role of glycosylation of the flagellum in *P. aeruginosa* infections, the molecular bias of glycosylation system. We will construct chimeric flagellins that will consist of glycosylated or nonglycosylated regions in the same genetic background. These mutants will be compared to the glycosylated a-type parent strain for virulence in animal models of infection. Next, flagellins with higher degrees of glycosylation will be compared to the PAK parent strain in an animal model to examine whether there is a relationship between virulence and degree of glycosylation. A "glycosylation island" that is present among *P. aeruginosa* flagellar genes will be examined in detail, to ascertain the roles of these genes in glycosylation. *Pseudomonas* homologues of genes involved in flagellar glycosylation in other bacteria will be mutagenized and examined for any for any effects on flagellin glycosylation, general protein glycosylation and LPS structure. There may also be accessory genes involved, therefore transposon mutagenesis of the a-type strain will be don't to find these. Lastly, site directed mutagenesis will be done on the potential glycosylation sites of the PAK flagellin to ascertain where glycosylation occurs and such mutants will be used to unambiguously provide evidence for a role of glycosylation in virulence. The results of these studies should provide information to assess the mechanism and role of glycosylation of the flagellum of *P. aeruginosa* in virulence and provide information about the relationship of flagellum glycosylation, general protein glycosylation and LPS. The existence of a central gene product involved in these could prove to be of great significance in controlling virulence.

Grant: 1R01AI047884-01A1
Program Director: LANG, DENNIS R
Principal Investigator: FIERER, JOSHUA
Title: Innate Immunity to Salmonella Infections
Institution: VETERANS MEDICAL RESEARCH FDN/SAN SAN DIEGO, CA
DIEGO
Project Period: 2001/03/01-2006/02/28

DESCRIPTION (provided by the applicant): Salmonella are one of the most important causes of gastrointestinal infections. The overall goal of our research is to understand how the innate immune response helps to protect against salmonella infections. The first aspect of innate immunity that we will study is PMNs. We previously reported that human epithelial cells make the potent chemotactic peptide IL-8 when invaded by salmonella, which implies that PMNs are important for the host defense against this pathogen. We then showed that PMNs are crucial for defense against salmonella that have virulence plasmids, but not those without virulence plasmids. This proposal focuses on the role of PMNs in protecting the intestine from salmonella infections, using a model of oral infection in mice. The role of PMNs will be established by making mice neutropenic with a monoclonal antibody (RB6-8C5) to the Ly-6G antigen that is specific for myelocytes. We will look for evidence that one or both chemokines are made in vivo in infected mice and in vitro using both cultured murine intestinal epithelial cells and colon organ culture. The second component of the innate immune response that we will investigate is lipopolysaccharide binding protein (LBP). We have confirmed the report by Jack et al (Nature, 389,742,1997) that LBP-deficient mice are more susceptible to salmonella infections, but the mechanism of action of LBP has not been elucidated in infection. We used mice that had the wild-type Nramp1 allele so are genetically resistant to salmonella infections. LBP deficient mice had lower levels of 2 ELR+ CXC chemokines. We will now study the role of CXC chemokines in host resistance to Salmonella by blocking the CXC receptor with antibody. The third goal is to determine whether complement mediated phagocytosis is critical for early resistance to salmonella infections. We will study the course of infection in mice that are deficient in C3 and determine whether neutropenia makes them more susceptible to infection. We will compare isogenic strains of salmonella that express either the group B or O LPS antigens in C3 deficient and neutropenic mice, in order to determine how the structure of the LPS affects virulence in mice.

Grant: 1R01AI047896-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: JOHNSON, RUSSELL C PHD
Title: HUMAN GRANULOCYTIC EHRLICHIOSIS: NORTH CENTRAL U.S.
Institution: UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN
Project Period: 2001/02/01-2006/01/31

DESCRIPTION: Human granulocytic ehrlichiosis (HGE) is an emerging tick-borne acute febrile illness. The agent of HGE is closely related to or identical to the horse pathogen Ehrlichia equi and is transmitted by Ixodes scapularis, the same tick that transmits the agents of Lyme disease and babesiosis. The HGE agent infects blood polymorphonuclear neutrophils resulting in neutropenia and a thrombocytopenia. HGE may be a mild to potentially fatal disease. HGE was first described in Minnesota and Wisconsin in 1994 and subsequently more than 400 cases have been described in the U.S. In contrast to Lyme disease which frequently occurs in children, the rates and severity of HGE increase with age. This age-specific incidence of HGE and our large aging population presents a potentially major public health problem with corresponding economic consequences. This emerging public health problem highlights the importance of early diagnosis and treatment in preventing complications of infection, and targeting of prevention and control studies to populations at highest risk. The research proposed in this application is designed to aid in achieving the above goals by analyzing the epizootiology of HGE in the North Central U.S. Our specific aims are: to investigate the hypothesis that white-tailed deer serve as a large mammal reservoir of the agent of HGE and to identify the small mammal reservoirs of the HGE agent.

Grant: 1R01AI047928-01A2
Program Director: RUBIN, FRAN A.
Principal Investigator: MCIVER, KEVIN S
Title: Analysis of MGA protein from the Streptococcus pyogenes
Institution: UNIVERSITY OF TEXAS SW MED DALLAS, TX
CTR/DALLAS
Project Period: 2001/09/30-2005/07/31

DESCRIPTION (provided by applicant): The group A streptococcus (Streptococcus pyogenes, GAS) is a bacterial pathogen of enormous medical importance to humans, causing a variety of disease syndromes that range in severity from minor to life-threatening. Mga is a DNA-binding protein of GAS that activates the transcription of several key virulence genes in response to changing environmental conditions, likely through interactions with other regulatory components in the cell. The Mga regulon encodes products essential for the survival of GAS in the host, including the antiphagocytic M protein, M-like immunoglobulin binding proteins, the secreted inhibitor of complement, a collagen-like protein, and a C5a peptidase. Thus, Mga provides an excellent model system to study global regulatory networks involved in GAS pathogenesis and how they may interact. However, we currently know very little about Mga, including what domains of the protein are critical for its function and how environmental signals control the Mga regulon. The specific aims of this project are: (1) To identify domains of Mga involved in DNA-binding and characterize their role in targeting specific promoters; (2) To determine a consensus Mga binding element for each of the known promoter sites through identification of specific Mga/nucleotide interactions; (3) To investigate whether domains of Mga interact directly with other bacterial components to transduce environmental signals (i.e., is Mga a two-component response regulator?); (4) To identify additional factors required for the environmental regulation of the Mga regulon and assess their role in global virulence regulation. An attractive feature of this proposal is our ability to study Mga both as a purified protein in vitro and as a native molecule in its GAS background. As such, we will be able to thoroughly address key questions of GAS pathogenesis associated closely with the environmental regulation of the Mga regulon. The overall objectives of this proposal are (A) to undertake a structure/function analysis of Mga and determine the mechanisms by which this key GAS virulence regulator contributes to streptococcal disease, and (B) improve our general understanding of regulatory pathways that broadly control virulence in these gram-positive pathogens.

Grant: 1R01AI047937-01A1
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: CALCUTT, MICHAEL J PHD
Title: Novel Integrative Genetic Elements of Mycoplasmas
Institution: UNIVERSITY OF MISSOURI COLUMBIA COLUMBIA, MO
Project Period: 2001/06/01-2004/05/31

DESCRIPTION (provided by applicant): Although horizontal gene transfer by plasmids and bacteriophages has long been known to disseminate key traits among bacterial populations, only recently has the contribution of mobile chromosomal elements been fully realized. Large chromosomal "islands" that confer pathogenic, symbiotic or metabolic traits have been described, some of which can be conjugated between bacteria. Other important integrated elements exist and the term CONSTIN has been proposed to describe those that are conjugal self-transmissible integrating elements. Studies of the distribution of these diverse elements, their ability to exchange genetic information and their potential to alter the phenotype of an organism by en bloc transfer of discrete gene functions, are still in their relative infancy. Some CONSTINs integrate site-specifically; others are less restricted in their choice of insertion site. The latter may profoundly influence chromosome structure and dynamics, and modulate gene function via mutational changes accompanying promiscuous integration. Recently, distinctive CONSTIN-like elements were identified in two pathogenic mycoplasmas, *M. fermentans* and *M. capricolum*. The two elements share features including the presence of conjugation-like genes, flanking direct repeats and a non-replicative extrachromosomal form. Importantly, most genes on these unusual elements lack recognizable homologs and a gene encoding a recognizable DNA integration enzyme is not present. It is anticipated therefore, that a "novel" enzyme performs this integration/excision function. Mycoplasmas typically occupy host niches for long periods during chronic infections in diverse vertebrates, and therefore may serve as important reservoirs for this "novel" element. This underscores the need to understand the dynamics of these elements both within chromosomes of mycoplasmas and in the context of the exchange of mobile gene pools within a population. Accordingly, the specific aims of this proposal are designed (i) to assess the distribution and genomic context of the *M. capricolum* element in host-related mycoplasmas; (ii) to test the hypothesis that the element is indeed capable of chromosomal integration, and (iii) to explore possible DNA-protein interactions within the termini of the element, as these are predicted to play a critical role in mobility. Completion of these goals will increase our understanding of the function of these novel elements and will provide a platform from which the long term objectives, of understanding their capacity for intra- or inter-species gene transfer, their contribution to the observed plasticity of mycoplasma genomes and their possible role in pathogenesis, can be explored.

Grant: 1R01AI047938-01A1
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: MILLER, SAMUEL I
Title: Pseudomonas aeruginosa lipid A
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2001/03/15-2006/02/28

Patients with cystic fibrosis (CF) suffer from chronic airway infections with the opportunistic pathogen *Pseudomonas aeruginosa* (PA), and experience worsening, irreversible lung injury leading to premature death. This injury is mediated by an inflammatory process that results, at least in part, from stimulation of the innate immune system by PA lipid A, the bioactive component of lipopolysaccharide (LPS). Preliminary data indicates that PA responds to the CF lung environment by synthesizing lipid A structures not present in clinical isolates from non- CF PA infections. This project will test the hypothesis that CF-specific lipid A structures promote resistance to innate immune mechanisms such as antimicrobial peptides and result in increased inflammatory responses. Key steps in the synthesis of CF-specific lipid A are hydroxylation, palmitoylation, and the synthesis and transfer of aminoarabinose. Genes encoding the relevant enzymes will be defined, and insertional mutants will be constructed and tested in assays of antimicrobial peptide resistance. Aerosolization of mutant whole bacteria or LPS into the murine airway or incubation in cell culture will be used to test the role of CF-specific lipid A modifications in inflammatory responses. CF-specific lipid A structures will be purified and their biological activity characterized. Finally, outer membrane proteins (OMPs) coordinately regulated with CF-specific lipid A will be identified by proteomic analysis, and the role of such OMPs in antimicrobial peptide resistance will be assayed in insertional mutants. These studies will provide insight into bacterial mechanisms that contribute to CF lung disease, including the role of lipid A modifying enzymes. Such enzymes may provide novel targets for the development of drugs to treat PA lung infections and their inflammatory consequences.

Grant: 1R01AI047999-01A1
Program Director: LANG, DENNIS R
Principal Investigator: DREYFUS, LAWRENCE A
Title: Molecular Analysis of the Cytolethal Distending Toxin
Institution: UNIVERSITY OF MISSOURI KANSAS CITY KANSAS CITY, MO
Project Period: 2001/07/01-2006/05/31

The cytolethal distending toxin (CDT) is a potent bacterial toxin produced by a growing list of unrelated bacterial pathogens including scattered isolates of *E. coli* and *Shigella* isolates, *Campylobacter* spp., *Haemophilus ducreyi*, *Actinobacillus actinomycetemcomitans*, and enteropathogenic *Helicobacter* spp. Initially, CDT was characterized by its capacity to induce massive cellular distension and cell death. Cells treated with CDT undergo an irreversible block in cell division caused by disruption of the cell cycle in G2. The specific events leading to CDT-mediated growth arrest parallel those following induction of the mitotic DNA damage checkpoint. CDT is the product of three genes designated *cdtA*, *cdtB*, and *cdtC*, which encode proteins with molecular masses of 30, 32, and 20 kDa. Genetic and biochemical evidence indicate that all three polypeptides are required for cellular intoxication. We recently reported that CdtB bears significance position-specific sequence relatedness to mammalian type I DNase. Mutational analysis indicates that the DNase-related active site residues in CdtB are required for biological activity. In preliminary studies show that purified CdtB possesses Mg²⁺-dependent DNase activity. We also present evidence indicating that CDT damages chromosomal DNA followed by activation of elements of the DNA damage checkpoint cascade. Although not toxic when added alone to cells, introduction of CdtB into cells results in the entire spectrum of CDT activities. We therefore hypothesize that CdtB mediates the cytolethal effects of CDT while CdtA and CdtC are required for cell binding and/or translocation of CdtB. In this application we propose to: 1) define the role of each of the three CDT polypeptides in cell binding and CdtB entry, 2) determine the mechanism by which CdtB traffics through the cell and translocates into nucleus (the apparent site of CdtB action, and 3) define the series of events leading to CDT-mediated growth arrest and death. Completion of these aims will provide new insights into the novel action of this potent bacterial toxin.

Grant:	1R01AI048052-01A2	
Program Director:	KORPELA, JUKKA K.	
Principal Investigator:	VOGEL, JOSEPH P	BS
Title:	Intracellular Growth of Legionella Pneumophila	
Institution:	WASHINGTON UNIVERSITY	ST LOUIS, MO
Project Period:	2001/09/30-2006/07/31	

The long term objective of this proposal is to understand how a Gram negative bacterial pathogen, *Legionella pneumophila*, is able to survive and replicate inside normally bactericidal phagocytic cells. This grant will focus on determining how *L. pneumophila* alters the endocytic pathway and prevents phagosome-lysosome fusion of host cells. The specific aims are to characterize the Dot apparatus by examining the role of two critical Dot proteins, DotB and DotL, in the assembly and activity of the Dot/Icm complex and to identify the substrate(s) exported by this complex into the macrophage host cell. The health relatedness of this proposal is two fold. First, establishing the mechanism used by *L. pneumophila* to survive and replicate inside macrophages will provide additional insight into how it causes disease and may reveal novel targets to be used for drug therapy. Second, since specialized secretion systems are commonly used by a variety of bacterial pathogens, knowledge gained about the *L. pneumophila* secretion apparatus is likely to be applicable to understanding the molecular mechanisms of virulence used by other pathogens, and could serve as the basis to prevent or treat a number of different diseases.

Grant: 1R01AI048067-01A1
Program Director: SAGER, POLLY R.
Principal Investigator: CRAIG, BRUCE A BA
Title: Calibrating the two Antimicrobial Susceptibility Tests
Institution: PURDUE UNIVERSITY WEST LAFAYETTE WEST LAFAYETTE, IN
Project Period: 2001/07/15-2003/06/30

To determine the susceptibility of an unknown pathogen to a specific drug, hospital laboratories perform either a drug dilution or disk diffusion test. Test/Drug specific breakpoints then classify the pathogen as either susceptible, intermediate or resistant to the drug. Because only one of these tests will be used in practice, it is imperative to have the two tests give similar classification results. It is the responsibility of subcommittees of the FDA and NCCLS to determine comparable breakpoints. Since the drug dilution test involves concentrations of the drug, its breakpoints are based largely on the pharmacokinetics and pharmacodynamics of the drug. Currently, disk diffusion breakpoints are determined using a modified version of the error-rate bounded method. This involves the creation of a scatterplot of test results for a wide range of pathogens and finding breakpoints which minimize the number of classification discrepancies. While this procedure is very fast and simple to implement, it does not adequately take into account the inherent variability of each test, the drug-specific relationship between the two tests, nor the underlying distribution of pathogens. As a result, the procedure's choice of breakpoints is very sample dependent. For this project, an alternative method of breakpoint determination will be developed which uses the same scatterplot of test results but explicitly account for the various factors of uncertainty using a hierarchical model. Probabilities from this model, which do not depend on the distribution of isolates, will then be used to determine appropriate breakpoints. While this procedure will be more time consuming to implement (particularly the inference component), it is believed that by accounting for the inherent test variabilities and underlying distribution of pathogens, much of the sample dependency will be eliminated giving more consistent and interpretable results.

Grant: 1R01AI048090-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: LEWINSOHN, DAVID M
Title: Recognition of Mtb-infected cells by CD8+ T lymphocytes
Institution: OREGON HEALTH & SCIENCE UNIVERSITY PORTLAND, OR
Project Period: 2001/07/01-2006/06/30

DESCRIPTION (the Applicant's Abstract): Tuberculosis remains an important clinical problem throughout the world. Protective immunity to tuberculosis depends upon the coordinated response of the cellular immune system. Vaccination strategies that have successfully elicited CD4+ T cells have not been uniformly successful in containing Mtb growth in animal models. Additionally, mice deficient in beta 2 microglobulin and hence MHC class I-dependent T cell responses are exquisitely sensitive to infection with Mtb. These data would argue that CD8+ T cells play an essential and distinct role in the host response to tuberculosis. Identification and characterization of human cytotoxic T cells is important for the understanding of immunity to tuberculosis, and hence may be crucial for the development of efficacious vaccines and improved therapeutic strategies. Work done by the PI and collaborators has previously established the existence of Mtb-specific CD8+ T cells in persons infected with Mtb. Using a sensitive IFN-gamma enzyme linked immunospot (ELISPOT) based limiting dilution analysis (LDA) to evaluate CD8+ T cells grown in response to Mtb-infected dendritic cells (DC), the majority of these cells from one latently-infected individual were found to be non-classically restricted in that they were neither HLA-A, B, or C nor CD 1 restricted. While in the minority, classically (HLA-Ia) restricted cells were also elicited, and the cognate antigen and its minimal peptide epitope defined. Important questions remain unresolved regarding the role of CD8+ T cells in the host response to infection with Mtb in several areas. First, the magnitude of classically restricted non-classically restricted, and CD 1 restricted responses following infection with Mtb is not known. This may be critical in distinguishing a successful (healthy, PPD positive; PPD+) from unsuccessful (tuberculosis) response to infection with Mtb. Second, the molecular basis for antigenic presentation to non-classically restricted CD8+ T cells is not known. Preliminary data are presented indicating that HLA-E may be the restricting molecule for these non-classically restricted cells. Third, prior work has relied upon peptides with predicted binding to HLA motifs to elicit classically restricted CD8+ CTL. One limitation of these peptide-based approaches is that it is difficult to ascertain whether these responses are primed by mycobacterial infection, or represent low-affinity cross-reactivity with another antigen. Similarly, it remains uncertain as to whether the peptides tested reflect dominant epitopes generated during the course of natural infection. Consequently, the question of whether or not antigens known to elicit CD4+ T cell responses also elicit CD8+ T cell responses remains unanswered. Finally, how these Mtb-derived proteins gain access to the HLA-Ia processing pathway remains an important and unresolved question in understanding the human host response to this important pathogen.

Grant: 1R01AI048103-01A1
Program Director: SAVARESE, BARBARA M.
Principal Investigator: KATZ, DAVID F PHD
Title: Biophysics of Microbicides for STD Prevention
Institution: DUKE UNIVERSITY DURHAM, NC
Project Period: 2001/08/01-2006/06/30

DESCRIPTION (Provided by Applicant): The goal of this project is to develop and apply a new methodology that characterizes deployment of microbicidal formulations in women, and that elucidates biophysical mechanisms which govern deployment - the spreading and retention of formulations over the intra-vaginal epithelial surfaces. The precise nature of deployment that is sufficient for prophylaxis against STD pathogens is unknown, and there is little knowledge of deployment characteristics of any formulation in women. This project will thus obtain unprecedented knowledge and data. A new intravaginal optical sensing device, developed by our laboratory, will be employed to quantitate formulation coating thickness distributions in women, including their dilution with local fluids and pH. A series of studies will contrast important biological and biophysical factors, e.g. time and motion after formulation application, cycle phase and simulated coitus - for a set of contemporary formulations, used as vaginal lubricants, contraceptives, and/or being considered as potential STD prophylactic materials. The results will have immediate relevance to a broad spectrum of basic biological and clinical studies in microbicide development. This project will also employ objective biophysical analysis, experimental and theoretical, to develop relationships between formulation properties and deployment characteristics. At present there is virtually no such knowledge, nor methodology to obtain it. Our approach is to develop a set of integrated in vitro experimental simulations of salient bio-fluid mechanical processes that produce formulation flow and retention within the vagina. For each such process we will also develop fluid mechanical theory that predicts biologically important endpoints (e.g. formulation spreading rates, layer thicknesses and properties) using measured values of formulation properties as inputs. Correspondence between theory and experiment will provide a means of physical validation of the in vitro methods. The final goal of the project will be to link the in vitro methods with the in vivo results. This will be a cross validation analysis, in which measures of deployment in vivo are related to predictions in vitro, first from the simulations and then from the mathematical models themselves. To the extent that the latter link is established, we will have developed an objective and accurate means of relating formulation properties directly to microbicidal function. To the extent that our in vitro methodology is accurate, it will provide an extremely useful and cost effective means of evaluating current formulations and designing new and improved ones. It will also provide unprecedented biophysical data relevant to mechanisms of STD infection in women.

Grant: 1R01AI048474-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: CONLAN, JOSEPH W PHD
Title: Acellular vaccines against *Francisella tularensis*
Institution: NATIONAL RESEARCH COUNCIL OF OTTAWA, ON
CANADA
Project Period: 2001/06/15-2006/04/30

DESCRIPTION (Provided by Applicant): The facultative intracellular bacterial pathogen, *Francisella tularensis*, can cause severe pneumonia and death following the inhalation of very small numbers of infectious particles. For this reason, *F. tularensis* is considered a primary biological warfare agent. Acquired host immunity against this pathogen is predominantly T-cell-mediated rather than humoral. An attenuated strain of *F. tularensis* is an effective live vaccine against virulent strains of the pathogen. However, this strain retains its virulence for mice, and might cause disease if administered to immunocompromised individuals. Thus, for mass-vaccination purposes, a defined fast-acting acellular vaccine would be preferable to the current live vaccine. Our institute has developed a novel vaccine delivery technology based on liposomes manufactured from the total polar lipids of various Archaeobacteria. These liposomes termed, archaeosomes, generate robust cell-mediated immune responses to model antigens entrapped within them, without the aid of any additional immune stimulants. Recently, we showed that a short peptide antigen of another intracellular pathogen, *Listeria monocytogenes*, packaged in archaeosomes, provides a high level of protective immunity against this pathogen in a murine listeriosis model after only a single vaccination. Because multiple studies indicate that the same host defenses are needed to combat *F. tularensis* and *L. monocytogenes*, it is likely that appropriate antigens of the former pathogen encapsulated in archaeosomes will provide effective acellular vaccines. This proposal will explore this possibility. It is expected that the findings from the proposed studies will be applicable to the development of acellular vaccines against other intracellular respiratory pathogens such as *Mycobacterium tuberculosis*, and *Chlamydia pneumoniae*.

Grant: 1R01AI048496-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: FICHT, THOMAS A PHD
Title: Improved Brucella Vaccine Strains
Institution: TEXAS AGRICULTURAL EXPERIMENT COLLEGE STATION, TX
STATION
Project Period: 2001/08/01-2004/07/31

DESCRIPTION (provided by applicant): Despite awareness of brucellosis for more than two millennia, identification and treatment of human illness is documented only within the last century. Reduction in animal disease has been used strategically to reduce human disease. Treatment of human infection relies upon antibiotic therapies, but relapse is not uncommon and with the advent of bioengineering, the ability to introduce antibiotic resistance into Brucella may negate the only method for direct treatment of human brucellosis. Brucellosis in humans can affect a number of different tissues, but is most typically associated with the lympho-histiocytic disease that if left untreated invades other tissues and can kill the host. Disease depends upon the ability of the organism to survive intracellularly, and includes persistence within professional phagocytic cells. Protective immunity in the host requires both the humoral and cellular responses, and although much effort has been invested in the development of subunit vaccines there has been little of success along these lines. The safety of currently available vaccine strains for human use is questionable, since these are often used to prevent abortion with less concern for protection against infection. The aim of the work proposed is the development of improved vaccines based on attenuated intracellular survival to minimize persistence of the organism while stimulating a protective immune response. Signature-tagged mutagenesis will be used to identify mutants of attenuated virulence in the mouse model and the defect in intracellular survival will be verified in vitro in human macrophages. The mouse model of infection will be employed, since survival and persistence of the pathogen in this model relies upon intracellular survival in macrophages. Similarities in survival of the organism, disease and organ involvement support the use of the mouse model for the study of human brucellosis. Vaccination in mice will be evaluated for protection against both intraperitoneal and oral challenge. Many of the gene products identified may serve as targets for new therapeutic regimens, but that is beyond the scope of the current proposal.

Grant: 1R01AI048507-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: BLISKA, JAMES B PHD
Title: Intracellular survival determinants of *Yersinia pestis*
Institution: STATE UNIVERSITY NEW YORK STONY STONY BROOK, NY
BROOK
Project Period: 2001/07/01-2004/06/30

DESCRIPTION (provided by applicant): *Yersinia pestis* is the agent of plague, an acute, often fatal bacterial infection that is transmitted by flea bite or aerosol. *Y. pestis* is considered to be a facultative intracellular pathogen. Previous data indicate that *Y. pestis* survives and replicates within macrophages during the first few hours of infection, while extracellular growth is predominant at later time points. The mechanism by which *Y. pestis* survives and replicates in macrophages is not known. The long-term objective of the proposed studies is to determine the molecular and cellular basis for intracellular survival of *Y. pestis* in macrophages. To achieve this goal we will (1) Characterize macrophage defenses that are important for limiting intracellular survival and replication of *Y. pestis*; and (2) Identify genes in *Y. pestis* that are important for combating intracellular defenses of macrophages. Understanding the intracellular survival mechanism of *Y. pestis* may aid the development of new ways to prevent or treat plague infections in the human population.

Grant: 1R01AI048526-01A1
Program Director: NEAR, KAREN A.
Principal Investigator: CHAISSON, RICHARD E
Title: Novel TB Prevention Regimens for HIV-Infected Adults
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 2001/09/07-2006/07/31

DESCRIPTION: (provided by applicant) Tuberculosis (TB) continues to kill approximately 2 million people per year, and is a major cause of HIV-related morbidity and mortality in developing countries. In addition, TB appears to cause a more rapid progression of HIV disease even when it is successfully treated, as active TB causes upregulation of HIV replication through several mechanisms. Although the World Health Organization has promoted a strategy of treatment of active TB as the principal weapon for TB control, accumulating evidence indicates that preventive therapy is required, particularly in countries with a high HIV burden. Both isoniazid (INH) and rifampin and pyrazinamide (RIF/PZA) have been shown to be effective in reducing the short-term incidence of TB in HIV-infected, tuberculin positive people, but the long-term benefit is not clear. Moreover, the implementation of TB preventive therapy programs in developing countries has been hindered by multiple factors. Establishment of clinical infrastructures to provide preventive therapy, concerns about adherence to treatment regimens, and a high likelihood of reinfection with subsequent increased risk of primary TB have been suggested as reasons not to implement preventive programs for HIV-infected populations. The purpose of this trial is to determine the effectiveness of three novel treatments on the risk of TB in a population of HIV-infected adults receiving clinical care and follow up in Soweto, South Africa. We will randomize 1141 adults with HIV infection and a reactive tuberculin skin test to receive weekly rifapentine and INH (RPT/INH) for 12 weeks, twice-weekly rifampin and pyrazinamide (RIFIPZA) for 8 weeks, continuous INH daily indefinitely (INH-C), or the internationally accepted standard of INH daily for six months (INH-6) for the prevention of TB. RPT is a rifamycin-S derivative with antimicrobial activity similar to rifampin, but with a longer half-life. RPT is efficacious in the treatment of non-HIV-related TB, and has been shown in animal models to be a highly promising agent for treating latent TB. We hypothesize that the increased tuberculocidal activity and programmatic advantages of supervised, once- or twice-weekly regimens with rifamycin-based combinations will be more effective than INH-6. We also hypothesize that a continuous course of INH will be more effective than INH-6 because elimination of latent TB will be more thorough and prophylaxis will provide ongoing protection against incident TB infection. By studying TB preventive therapy in a setting of comprehensive HIV care for adults in a developing country setting, we will be able to generate critically important data on alternative therapeutic options for TB control among HIV-infected people that will be applicable throughout the world.

Grant: 1R01AI048551-01A1
Program Director: LANG, DENNIS R
Principal Investigator: WILKS, ANGELA PHD
Title: Heme Acquisition in Shigella Dysenteriae.
Institution: UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MA
SCHOOL
Project Period: 2001/06/01-2006/04/30

DESCRIPTION (Provided by Applicant): The objective of the current proposal is to identify and characterize at a functional and structural level the proteins involved in heme transport in bacterial pathogens. Pathogenic bacteria require iron for their survival and growth and the ability to acquire this element is linked in part to their virulence. The major source of iron within the body is complexed in heme and heme-containing proteins. Pathogenic bacteria have developed sophisticated mechanisms by which they acquire iron from the hosts heme and hemeproteins. The current increase in the occurrence of antibiotic-resistant strains has created a need to develop alternative strategies to target such pathogens. Structural and functional characterization of proteins involved in heme transport and storage should make it possible to develop specific inhibitors. The expression and purification of the outer-membrane heme receptor (HutA) and periplasmic heme-binding protein (ShuT), will allow biochemical characterization of the heme transport proteins in gram-negative pathogens. The study will be directed towards two specific goals: first, the nature of heme binding to the receptor and transport proteins, and second, the mechanism of heme uptake. Site-directed mutagenesis together with physical techniques such as optical absorption, fluorescence, MCD and resonance Raman spectrophotometry will provide information on the structural features required for heme-binding and transport. The substrate-specificity of the outer-membrane receptor (HutA) and the periplasmic binding protein (ShuT) will be determined with substituted porphyrins, with the premise that such data may provide templates for the development of small molecule inhibitors. The expression of HutA in a heme deficient strain of E. coli allows us to directly monitor heme uptake as a function of cell viability and provides a direct method of determining heme transport in site-directed mutagenesis studies. In addition the coexpression of a bacterial heme oxygenase with the HutA receptor will be developed as a rapid colorimetric screen for future site-directed mutagenesis and inhibitor screening. The redox nature of heme transport will also be investigated with redox inactive non-iron metalloporphyrins such as Co and Zn-protoporphyrin IX. Once an understanding of the mechanism of heme transport in bacterial pathogens has been established the stage will be set for future design of selective inhibitors of heme transport.

Grant: 1R01AI048583-01
Program Director: KORPELA, JUKKA K.
Principal Investigator: KING, GLENN F PHD
Title: TOPOLOGICAL REGULATION OF PROKARYOTIC CELL DIVISION
Institution: UNIVERSITY OF CONNECTICUT SCH OF FARMINGTON, CT
MED/DNT
Project Period: 2000/01/01-2005/12/31

DESCRIPTION (Adapted from applicant's abstract): Bacterial cell division is a complex differentiation process achieved with remarkable fidelity. The division septum is formed at mid-cell so that cytosolic components are equi-partitioned into daughters. Selection of the correct division site in *E. coli* is accomplished by the cooperative action of a division inhibitor (MinC-MinD) and a topological specificity factor (Min E). We still do not understand their mechanism of action. A key to achieving such understanding will be determining the 3-dimensional structure of these proteins and the molecular details of their interactions. Specifically, it is planned to develop in vitro interaction assays to map regions of these proteins responsible for interactions with cognate partners, as well as the interaction of MinC with the cell division inhibitor DicB. Site directed mutagenesis as well as insertion and deletion mutants will be used to map regions of interaction. The phenotypic effects of these mutants will be examined and fluorescence microscopic examination will localize the cellular location of MinE point mutants lacking topological specificity. Limited proteolytic digestion will determine the core interacting domains of each protein. A combination of NMR and X-ray diffraction will then be used to determine structures of individual proteins, domains and protein complexes. The protein structures will be used for the structure based design of novel antibacterial agents.

Grant: 1R01AI048593-01
Program Director: MILLER, MARISSA A.
Principal Investigator: BRENNAN, RICHARD G
Title: X-RAY STUDIES ON BACTERIAL MDR REGULATORS
Institution: OREGON HEALTH & SCIENCE UNIVERSITY PORTLAND, OR
Project Period: 2001/03/01-2005/02/28

DESCRIPTION: (Verbatim from the Applicant's Abstract) The emergence of bacterial multidrug resistance (MDR) poses a serious threat to human health. One key factor underlying MDR is membrane bound transporters that extrude multiple, chemically diverse drugs from the bacterial cell. The structural mechanism by which these proteins recognize dissimilar drugs is completely unknown, primarily because they are integral membrane proteins and thus more difficult to purify. Bacteria also have a second class of multidrug binding proteins that is central to their multidrug resistant phenotypes. These cytosolic proteins are transcription regulators of the multidrug transporter genes. One regulator from *Bacillus subtilis* is BmrR. BmrR dramatically increases transcription of the multidrug transporter gene, *bmr*, only after binding drugs that are Bmr substrates but have invaded the cytosol. Thus, BmrR acts as a second line of defence against drugs from reaching their cellular targets. Structures of BmrR-Drug and BmrR-DNA+Drug complexes will also reveal the transcription regulation mechanism of the MerR family member, the class to which BmrR belongs. A second multidrug binding regulatory protein is QacR from *Staphylococcus aureus*. QacR represses the *qacA* and multidrug transporter gene and belongs to the TetR/CamR family. Drugs, which are also substrates of the QacA transporter, induce QacR and derepress the *qacA* gene thereby providing the bacterium with the more transporters to fend off potentially lethal drug doses. Structural studies will unveil the underpinnings of the multidrug binding and transcription repression mechanisms of QacR. Interestingly, QacR and BmrR display overlapping drug binding specificities and structures of their same-drug complexes will reveal the similarities and differences of their multidrug binding mechanisms. This grant proposal has four specific aims. To crystallize and determine the structures of the C-terminal, multidrug binding domain of BmrR, the so named BRC, bound to a number of drugs that display a wide range of binding affinities. BRC offers the advantages of high resolution, which will greatly aid the analysis of the drug binding mechanism of BmrR. To crystallize and determine the x-ray structures of BmrR-Drug-DNA and BmrR-DNA complexes. To crystallize and determine the x-ray structures of the *B. subtilis* global MD regulator, MtaN and its DNA complexes. To crystallize and determine the x-ray structures of QacR-drug and QacR-DNA complexes. The broad goals of this work are to provide a complete understanding of the mechanisms of multidrug binding by BmrR, MtaN and QacR and gene regulation of these MerR and TetR/CamR family members. These data will be key to the future structure-based drug design of novel drugs against pathogenic bacteria.

Grant: 1R01AI048611-01A1
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: GENCO, CAROLINE A PHD
MICROBIOLOGY:MICROBIO
OGY-UNSPEC
Title: Gonococcal Fur Regulon--Link to Pathogenesis
Institution: BOSTON MEDICAL CENTER BOSTON, MA
Project Period: 2001/07/15-2006/06/30

DESCRIPTION (provided by the applicant): In most Gram-negative pathogens, genes involved in iron acquisition and virulence are transcriptionally regulated by the availability of iron through the ferric uptake regulator protein, Fur. The etiological agent of the sexually transmitted disease gonorrhea, *Neisseria gonorrhoeae*, produces a number of iron regulated proteins which are utilized for iron transport and expression of these proteins is thought to be under the control of Fur; however, the role of Fur in controlling expression of iron transport genes or virulence factors in *N. gonorrhoeae* is not well defined. This application proposes to further our understanding of the regulation of genes involved in iron transport and virulence by the transcriptional regulator protein Fur, and to define the role of the Fur-regulon in *N. gonorrhoeae* pathogenesis. Our hypothesis is that Fur controls the expression of a number of genes in addition to iron transport genes that are required for the virulence of *N. gonorrhoeae*. The specific aims of this proposal are as follows: Aim 1. To identify the minimal essential gonococcal nucleotide sequence for Fur binding and characterize the interactions of gonococcal Fur with iron-regulated promoters. Aim 2. To isolate and characterize iron-independent mutants of gonococcal Fur. Aim 3. To define the Fur regulon in *N. gonorrhoeae*. Aim 4. To define the role of the gonococcal Fur regulon in pathogenesis. These studies are based on the premise that gonococcal Fur binds to a unique and specific array of DNA sequences within the promoter regions of a number of Fur-regulated genes. This allows gonococcal Fur to function as a general global regulator controlling the expression of numerous genes in *Neisseria*. The intracellular iron concentration and the variability and extension of sequences targeted by Fur may cause a wide range of responses. Therefore, there may be many genes that are regulated by Fur that are unidentified. The results obtained in these studies will enable us to define the binding of gonococcal Fur to gonococcal Fur-regulated promoters, to identify additional genes which are regulated by Fur in *N. gonorrhoeae*, to define the mechanisms of Fur mediated regulation, and to correlate this with the pathogenic potential of *N. gonorrhoeae*.

Grant: 1R01AI048660-01A1
Program Director: KORPELA, JUKKA K.
Principal Investigator: CHURCHILL, MAIR E PHD
Title: Structural studies of bacterial quorum sensing regulator
Institution: UNIVERSITY OF COLORADO DENVER/HSC DENVER, CO
AURORA
Project Period: 2001/07/01-2006/05/31

DESCRIPTION (provided by applicant): Persistent bacterial infections are a major cause of death in cystic fibrosis patients and immune-compromised individuals. A number of gram-negative bacteria including *Pseudomonas aeruginosa*, a major pathogen in cystic fibrosis, cause infections that are difficult to treat because the bacteria form a "biofilm community" that renders them less sensitive to traditional antibiotics. Quorum sensing, mediated by acylhomoserine lactone (AHL) signaling molecules, regulates pathogenesis and biofilm formation in *P. aeruginosa*. Therefore, understanding the molecular basis of quorum sensing is a high priority in the development of novel anti-bacterial agents. The long term goal of this project is to extend the understanding of the quorum-sensing system to the atomic level to develop a detailed description of the mechanisms that control bacterial pathogenesis. The main focus of this proposal is the class of enzymes that produce the AHL signal, AHL-synthases, because bacteria lacking the AHL signal fail to become pathogenic or form stable biofilms. Although there are models of the mechanism of action of the AHL-synthases, there are currently no structures of any AHL synthase. High resolution structural information is absolutely essential for fully understanding the mechanism of AHL synthesis and will provide the basis for future structure-based inhibitor design for development of novel therapeutics. The specific aims for this project are: (I) determine the high resolution crystal structure of the *Pantoea stewartii* subsp. *Stewartii* AHL-synthase (EsaI) to understand its function, mechanism, and relationship to other enzymes that utilize similar substrates. Perform mutagenesis, binding and kinetics experiments with EsaI to better understand the catalytic mechanism and substrate specificity. (II) Study the *P. aeruginosa* AHL-synthase, LasI, using structural and biochemical techniques to understand how specificity of AHL production is determined. (III) Establish whether the AHL-synthase homologues in divergent organisms produce a homoserine lactone signal using mass spectrometry and activity assays. Study the structures and mechanisms to determine similarities to other AHL synthases.

Grant: 1R01AI048683-01
Program Director: LANG, DENNIS R
Principal Investigator: MILLER, SAMUEL I
Title: SALMONELLA PATHOGENICITY ISLAND 2 EFFECTOR PROTEINS
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2001/01/15-2005/12/31

DESCRIPTION (Adapted from the Applicant's Abstract): Salmonella are facultative intracellular pathogens which cause significant diseases in humans and animals. These organisms cause several disease syndromes, including enteric (typhoid) fever, gastroenteritis, bacteremias and focal infections. This grant proposes to study a murine infection with *S. typhimurium* and infection of macrophages and cultured epithelial cells with *S. typhimurium* and *S. typhi*. A set of virulence genes, termed Salmonella translocated effectors, that are translocated across the phagosome membrane into the eukaryotic cell cytoplasm by a type III secretion system encoded on the Salmonella pathogenicity island II will be studied. This grant proposes to further define these proteins and to study in molecular detail their role in virulence.

Grant: 1R01AI048689-01
Program Director: KORPELA, JUKKA K.
Principal Investigator: HULTGREN, SCOTT J PHD MICROBIOLOGY
MOLECULAR BIOLOGY
Title: PATHOGENIC FIBER FORMATION IN BACTERIA: STRUCTURAL BASIS
Institution: WASHINGTON UNIVERSITY ST. LOUIS, MO
Project Period: 2001/02/01-2006/01/31

DESCRIPTION (Verbatim from Applicant's Abstract): The biogenesis of diverse fibrous organelles by Gram-negative bacteria plays a critical role in the pathogenesis of many diseases. Uropathogenic E. coli assembles P and type 1 pili on their surfaces via the conserved chaperone-usher pathway. These pili mediate attachment to host tissues, a key early event in the development of disease. Pilus subunits have immunoglobulin-like (Ig) folds that lack their canonical C-terminal b-strand. The Ig-like periplasmic chaperone transiently donates a b-strand to complete the fold of the subunit, via a mechanism termed donor strand complementation. Subunits do not fold in the absence of a chaperone and are proteolytically degraded. During pilus assembly, the Ig fold of a subunit is thought to be more permanently completed by the exposed N-terminal extension of its neighbor, which displaces the chaperone in a mechanism termed donor strand exchange. Pilus subunits with complete Ig folds will be generated by genetically linking the relevant N-terminal extension to the C-terminus of the subunit to create stable, monomeric donor strand complemented (Dsc) subunits. Dsc subunits will be tested for their ability to fold in the absence of a chaperone to determine the role of the missing strand in subunit folding. Dsc and N-terminal deleted (Ntd) subunits, which have their N-terminal extensions removed and therefore cannot interact with other subunits, will be used with x-ray crystallography and in vivo and in vitro assembly systems to elucidate the structural basis of donor strand exchange. Stable Dsc adhesins will be crystallized with their saccharide receptors to determine the structural basis of microbial colonization and will be tested as vaccines to treat and prevent urinary tract infections. Gram-negative pathogens also assemble surface fibers termed curli via the nucleation-precipitation pathway. Curli share the diagnostic properties of amyloid fibers, which characterize a variety of human diseases, including Alzheimer's disease. The nucleation, formation, and structure of amyloid-like curli fibers will be studied. These studies will reveal general principles that govern the fundamental processes of protein folding and organelle biogenesis, shed light on bacterial attachment and its role in pathogenesis, and contribute to the development of new methods to treat and prevent a variety of diseases.

Grant: 1R01AI048696-01
Program Director: MILLER, MARISSA A.
Principal Investigator: FRALICK, JOE A PHD
Title: GENETIC ANALYSIS OF THE TOLC-VCEAB MDR EFFLUX PUMP
Institution: TEXAS TECH UNIVERSITY HEALTH SCIS LUBBOCK, TX
CENTER
Project Period: 2001/02/01-2005/01/31

DESCRIPTION (Adapted from applicant's abstract): The emergence of antibiotic-resistance among bacterial pathogens is becoming a serious and growing threat to human health and welfare. This resistance is due, in part, to the recently discovered multiple drug resistant (MDR) efflux pumps, which can handle a wide range of structurally different compounds. Moreover, these MDR systems can be amplified in resistant cells and can shift or expand their substrate profiles with mutation making them a major threat to drug therapy. Today we face the frightening possibility that many, if not all pathogenic bacteria may soon become resistant to all known antibiotics. Hence, understanding the structures, functions and mechanism of action of these efflux pumps will be important in the design of new drugs which can inactivate or circumvent the action of these MDR pumps. The MDR efflux pump in Gram-negative bacteria is composed of three different protein species, a cytoplasmic membrane transporter protein, a periplasmic fusion protein, and an outer membrane efflux protein. During the functioning of this pump these proteins form a complex, which translocates its substrate across the two membranes of the cell envelope and into the surrounding environment. TolC, which has relatives in most Gram-negative bacteria, is a broad-based outer membrane efflux protein, which interacts with a variety of protein export pumps and MDR efflux pumps of *Escherichia coli*. Understanding TolCs' structure-function relationship with such pumps should provide novel and important insights into these bacterial MDR systems. To ascertain such information we propose to utilize a large collection of TolC mutants to map the structure-function interactions of TolC with a specific MDR efflux pump, VceAB. These studies will entail physiological, biochemical and genetic analysis of the TolC-VceAB interactions, which may eventually lead to the design of novel and effective anti-microbial agents.

Grant: 1R01AI048704-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: RUBIN, ERIC J PHD
Title: Virulence Factors in Mycobacteria
Institution: HARVARD UNIVERSITY (SCH OF PUBLIC BOSTON, MA
HLTH)
Project Period: 2001/07/01-2006/05/31

DESCRIPTION (provided by the applicant): Tuberculosis infects much of the world's population and is responsible for millions of deaths annually. The causative organism, Mycobacterium tuberculosis, has been recognized for over a century, but little is known about the molecular mechanisms used by this bacterium to cause disease. I propose to use three methods we have recently developed in conjunction with the M tuberculosis genomic sequence to determine which genes are required by M tuberculosis to survive both in vitro and in vivo. First, we have developed a new transposon to perform saturating mutagenesis in M tuberculosis. Second, we have made a DNA microarray with which we can measure hybridization to each M tuberculosis open reading frame. Third, we have developed transposon junction hybridization (TJH), a method for mapping the sites of transposon insertions in large pools of mutants using a DNA microarray. We propose to use TJH to compare the genes required for M tuberculosis in vitro growth with those needed to survive in an animal. We will use a variation of TJH, differential length hybridization, to identify the complete set of genes that are essential for growth in defined media. We will also sequence several thousand clones from an insertion mutant library to produce a bank of defined mutants. This will allow us to test individual strains that contain mutations in candidate virulence genes identified by TJH. Since pathogens coordinately regulate expression of virulence genes, we will focus on regulatory genes required for infection and determine which downstream genes they control. This will enable us to identify both genes required for survival and for causing disease. Identification of genes important in infection should lead to the development of new strategies of tuberculosis treatment and prevention.

Grant: 1R01AI048708-01A1
Program Director: LANG, DENNIS R
Principal Investigator: FRANCO-MORA, AUGUSTO A MS ZOOLOGY
NEC:MICROBIOLOGY
Title: Molecular Evolution of Enterotoxigenic B fragilis
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 2001/06/01-2005/04/30

DESCRIPTION (provided by applicant): Enterotoxigenic *Bacteroides fragilis* (ETBF) strains, which produce a 20 kDa zinc metalloprotease toxin (BFT), have been associated with diarrheal disease of animals and young children. Studying a collection of ETBF and non-toxigenic *B. fragilis* (NTBF) strains, we found that: 1) The bft gene and a second metalloprotease gene (mph) are contained in a ca. 6-kb pathogenicity island (termed *B. fragilis* pathogenicity island or BfPAI). 2) The BfPAI is integrated between genes, which encode proteins sharing significant homology to mobilization proteins encoded by *Bacteroides* spp. mobilizable plasmids. 3) The G+C content of the mobilization genes flanking the BfPAI (49 percent) differ substantially from those of the BfPAI (35 percent) and the rest of the *B. fragilis* DNA (42 percent), indicating that the BfPAI is contained in an additional foreign genetic element, 4) There are three major populations of *B. fragilis* strains: pattern I strains, containing the BfPAI and flanking element, all are ETBF strains; pattern H strains, lacking the BfPAI and flanking element, all are NTBF strains; and pattern III strains, containing the flanking element but lack the BfPAI, all are NTBF strains, and 5) The BfPAI and its flanking regions are necessary to bft expression. Based on these results, we hypothesize that the BfPAI and flanking regions are important to the pathogenesis of ETBF strains, and that ETBF strains evolved by acquisition of the flanking element and the BfPAI. The long-range goal of this project is to investigate the molecular evolution of ETBF strains and to further understand ETBF disease pathogenesis. The specific aims of this proposal are: 1) To characterize the BfPAI and its flanking region; and 2) To investigate the molecular evolution of ETBF strains. Our results will advance our understanding of the molecular pathogenesis of ETBF infections and will serve to delineate how ETBF originated during evolution.

Grant: 1R01AI048769-01
Program Director: SAVARESE, BARBARA M.
Principal Investigator: ROCKEY, DANIEL D PHD
Title: CHLAMYDIA TRACHOMATIS INCA MUTANTS
Institution: OREGON STATE UNIVERSITY CORVALLIS, OR
Project Period: 2001/03/01-2006/02/28

DESCRIPTION (Adapted from the Applicant's Abstract): *C. trachomatis* variants have been described that are incapable of undergoing inclusion fusion in cell culture. These variants represent 1-2 percent of clinical isolates in Seattle and have been defined as lacking detectable IncA, a chlamydial protein that localizes to the inclusion membrane. Twenty-six independent incA mutant isolates have been sequenced and organized into several distinct categories. The overall goal of the proposed research is to identify distinctions between wild type and non-fusogenic strains and to exploit differences that are defined to better understand chlamydial development and pathogenesis. Experiments are planned to examine molecular and cell biology as well as clinical manifestations of the mutant chlamydiae. Each variant will be compared with matched wild-type controls. In the first aim molecular analyses will be done to determine mechanisms responsible for loss of expression of IncA and possibly other Inc's. In the second aim, growth and development of non-fusogenic strains will be studied in cell culture models. Finally, in the third aim the clinical relevance of the non-fusogenic phenotype will be determined using a retrospective case-control analysis and a monkey model of chlamydial infection. Study of these natural mutants will lead to a better understanding of chlamydial growth, development and pathogenesis.

Grant: 1R01AI048796-01
Program Director: KLEIN, DAVID L
Principal Investigator: JANOFF, EDWARD N MD
Title: MUCOSAL DEFENSE BY IGA AGAINST S PNEUMONIAE
Institution: UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN
Project Period: 2001/03/01-2006/02/28

DESCRIPTION (adapted from the investigator's abstract): *Streptococcus pneumoniae* is a common invasive mucosal pathogen. Local and systemic manifestations range from asymptomatic colonization, to sinusitis and otitis media, to community-acquired pneumonia, bacteremia, and meningitis, resulting in up to 40,000 deaths/year in the US alone. The local host and bacterial factors that determine whether *S. pneumoniae* causes no disease, mucosal disease, or invasive disease are not well-characterized. We propose to characterize the mechanisms of control of pneumococcal infections operative at the initial steps in their pathogenesis from nasopharyngeal colonization to aspiration into upper respiratory mucosae and alveoli, prior to alveolar injury and invasion into tissue and blood. We have shown that IgA reactive with pneumococcal capsular polysaccharide (PPS) mediates killing by neutrophils by both complement-dependent and -independent conditions. IgA and IgG may both contribute to defense of the lower respiratory tract by initiating killing by macrophages and neutrophils and by inhibiting adherence to epithelial cells. We will characterize the molecular and biochemical features of PPS-specific Ig which determine their functional role, their effective interactions with local phagocytic cells, as well as the adaptive mechanisms of the organism which may subvert these protective mechanisms. Hypotheses: (1) Molecular and biochemical characteristics of capsule-specific IgA and IgG (e.g., mutation rates of immunoglobulin genes (VH), monomeric, polymeric, and secretory structure of IgA, avidity, and pattern of IgA glycosylation) determine their functional efficiency to inhibit adherence and mediate killing by phagocytes. (2) Bacterial virulence factors (e.g., IgA1 protease, choline-binding protein A, and phase variation) subvert the ability of PPS-specific IgA to control *S. pneumoniae*. (3) The local host environment (e.g., complement, C-reactive protein) determines the phagocytes' ability of IgA and IgG to initiate killing of *S. pneumoniae*. The specific objectives of this proposal are designed to illuminate the unique features of the local host-pathogen interaction and defense against these extremely common and serious mucosal infections with *S. pneumoniae* in children and adults.

Grant: 1R01AI048825-01
Program Director: TSENG, CHRISTOPHER K.
Principal Investigator: MYERS, ANDREW G PHD
Title: A PRACTICAL SYNTHETIC ROUTE TO THE TETRACYCLINES
Institution: HARVARD UNIVERSITY CAMBRIDGE, MA
Project Period: 2001/01/01-2005/12/31

DESCRIPTION: (Principal Investigator's Abstract) The objective of this proposal is to develop the first practical, efficient, and enantioselective laboratory synthetic route(s) to the tetracycline antibiotics. The goal is to devise a route of twelve or fewer synthetic steps, perhaps as few as eight steps, beginning with benzoic acid as a starting material. A constraint that the synthetic route be versatile is also imposed, allowing for the introduction of substantive structural variability at a late stage, particularly within the C and D rings of the tetracycline structure, where prior research has shown that there is great opportunity for antibiotic development. Synthesis of a wide range of new tetracyclines for evaluation as improved antibiotics and, potentially antitumor agents is proposed. A particular focus is the development of effective antibiotics against tetracycline-resistant microorganisms. Adaptation and/or modification of these synthetic routes to target new tetracycline structures with antitumor activity, such as SF-2575 (TAN-1518 X) will also be attempted.

Grant: 1R01AI048856-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: CABELLO, FELIPE C MD OTHER AREAS
Title: GENETIC APPROACHES TO VIRULENCE IN B. BURGDORFERI
Institution: NEW YORK MEDICAL COLLEGE VALHALLA, NY
Project Period: 2001/02/01-2006/01/31

DESCRIPTION: (adapted from the applicant's abstract): *Borrelia burgdorferi* is an in vitro culturable bacterium that is the cause of Lyme disease. Its small genome contains approximately 1,000 chromosomal genes and 400 plasmid genes. Despite knowledge of the complete DNA sequence of the *B. burgdorferi* genome, identification and characterization of unique in vivo expressed *B. burgdorferi* virulence determinants has been delayed by the lack of expeditious and efficacious genetic systems in *Borrelia*. We have developed a genetic system in *B. burgdorferi* that consists of the extrachromosomal cloning vector, pGKI2, enhanced green fluorescent protein as a potential reporter gene, and resistance to erythromycin, kanamycin, and other antibiotics as selective markers. We have also been able to show that the *bmp* gene cluster is highly conserved among *B. burgdorferi sensu lato* strains, and that the genes of this cluster undergo environmentally modulated differential expression suggesting a potential role in virulence for these genes. The experimental protocol we propose is based on our preliminary work and framed by two hypotheses: 1) efficient molecular genetic systems can be developed for *B. burgdorferi*, and 2) the role of the *bmp* gene cluster in *B. burgdorferi* biology and virulence can be ascertained using these systems. With the long-term aim of identifying *B. burgdorferi* virulence determinants and improving our understanding of their in vivo expression and regulation, we propose the following Specific Aims: 1) Continue development and improvement of an extrachromosomal cloning system for *B. burgdorferi*, 2) isolate and complement *B. burgdorferi* *bmpD*, *bmpC* and *bmpA* null mutants to determine the possible role of these genes in *B. burgdorferi* virulence in in vitro and in vivo model systems of infection; and 3) characterize promoters and regulatory DNA sequences of *bmpC* using transcriptional fusions with enhanced green fluorescent protein (EGFP) and fluorescence-activated cell sorting (FACS). We expect these experiments will permit the extension of the molecular Koch's postulates to the characterization of unique and specific molecular virulence determinants of *B. burgdorferi*.

Grant: 1R01AI048917-01
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: PIER, GERALD B PHD MICROBIOLOGY, OTTAWA
Title: VIRULENCE AND IMMUNITY TO MUCOID P. AERUGINOSA
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 2001/01/01-2003/12/31

DESCRIPTION: (Adapted from applicant's abstract): The major goals of this project are to understand basic aspects of virulence and host immunity to mucoid strains of *P. aeruginosa* that are major causes of morbidity and mortality in cystic fibrosis (CF) patients. About 2500 new babies with CF are born each year worldwide, and by adolescence 80 per cent of them will become chronically infected with mucoid *P. aeruginosa*. Clinical data clearly show that the decline in pulmonary function and ultimate early mortality that occurs in *P. aeruginosa*-infected CF patients is due to mucoid *P. aeruginosa* infection and not to any other pathogen, including non-mucoid *P. aeruginosa*. Therefore, preventing new infections or treating established infections with immunotherapeutic agents that target mucoid *P. aeruginosa* should have a major impact on the health of CF patients. The primary hypotheses to be evaluated are that chronic mucoid *P. aeruginosa* infection is dependent on elaboration of an acetylated version of the mucoid exopolysaccharide (MEP) surface antigen, and that antibodies to the acetylated epitopes on MEP confer protection from chronic infection. A key component of this work will use our ability to initiate and establish a chronic mucoid *P. aeruginosa* infection in transgenic CF mice, a tool that, to date has eluded other researchers using these mice. The first hypothesis will be evaluated using in vitro studies to ascertain the ability of the acetylated MEP to confer resistance on mucoid *P. aeruginosa* to opsonic killing as well as in in vivo studies in CF mice. In the mice we will evaluate the ability of strains with differing abilities to acetylate MEP to establish and maintain a chronic colonization. The second hypothesis will be evaluated by production of conjugate vaccines composed of MEP with differing levels of acetylation, prepared by chemical manipulations. The conjugate vaccines will be evaluated for their immunogenicity in mice and rabbits and for their in vitro characteristics in regard to their ability to elicit antibodies that mediate opsonic killing of a multitude of mucoid *P. aeruginosa* strains. Next the vaccine(s) with the best ability to elicit high titers of broadly-reactive opsonic antibody will be tested in transgenic CF mice, using both active vaccination to prevent infection and passive therapy of established infection. In addition, we will test our proposed hypothesis that the presence of pre-existing, non-opsonic antibodies to non-acetylated epitopes on MEP in CF patients prior to the onset of mucoid *P. aeruginosa* infection interferes with the ability to mount a protective, opsonic response to the acetylated epitopes. Finally, we will evaluate the potential of some recently prepared human monoclonal antibodies to MEP to reduce the levels of mucoid *P. aeruginosa* in the lungs of infected CF mice. The results of this work should further our insights into the pathogenesis of mucoid *P. aeruginosa* infection, and promote development of effective vaccines and passive therapeutic reagents to prevent and treat mucoid *P. aeruginosa* infections in CF patients.

Grant: 1R01AI048935-01
Program Director: KLEIN, DAVID L
Principal Investigator: LIPSITCH, MARC PHD
Title: VACCINATION AND THE EVOLUTIONARY DYNAMICS OF PNEUMOCOCCI
Institution: HARVARD UNIVERSITY (SCH OF PUBLIC BOSTON, MA
HLTH)
Project Period: 2001/02/01-2006/01/31

Antigenically diverse pathogens such as pneumococci present novel evolutionary challenges for vaccine design. The use of vaccines directed against variable antigens can cause, and has caused, increases in pathogen types not carrying the antigenic variants included in the vaccine. this evolutionary response of the population of streptococcus pneumonia (pneumococcus) to vaccine - induced selective pressure, known as "serotype replacement," may in turn cause a shift in patterns of disease. serotype replacement may be either beneficial or harmful, depending on the ability of the replacing types to cause disease. The research proposed here is to ascertain is designed to ascertain the biological mechanisms underlying the observed population biology of pneumococci in unvaccinated populations and to use this knowledge to predict how the population will respond when vaccines are widely used. Experimental models of pneumococcal carriage in mice and analysis of epidemiological data will be used to characterize the ecological mechanisms underlying pneumococcal diversity and population biology, and these mechanisms will be incorporated into a mathematical model of pneumococcal carriage and transmission. The predictions of this model will be using molecular epidemiological studies of pneumococcal isolates from community randomized trial of the pneumococcal conjugate vaccine. the specific aims are: 1) to characterize quantitatively the population- biological interactions between pneumococcal strains in a laboratory mouse model of intra nasal carriage. 2) to characterize the development of antibody-mediated immunity due to natural pneumococcal carriages and the impact of this immunity on dynamics of pneumococci, to use these models to identify the mechanisms underlying existing patterns of the transmission mathematical models by characterizing the changes in pneumococcal populations following vaccination, using molecular epidemiological typing methods.

Grant: 1R01AI049139-01A1
Program Director: NEAR, KAREN A.
Principal Investigator: OBERHELMAN, RICHARD A MD
Title: Diagnostics for AIDS-Related Pediatric TB, Peru
Institution: TULANE UNIVERSITY OF LOUISIANA NEW ORLEANS, LA
Project Period: 2001/09/30-2005/07/31

DESCRIPTION: (provided by applicant) Tuberculosis is the major infectious cause of mortality among AIDS patients in the developing world, and HIV infection has been shown to increase mortality from tuberculosis five-fold in parts of Sub-Saharan Africa. Increasingly, HIV-infected children in developing countries are becoming infected with *Mycobacterium tuberculosis* (Mtb) and dying at an early age, presenting new dilemmas that differ from those facing adults with HIV-Mtb coinfection. The diagnosis of pediatric TB is complicated by inefficient and expensive methods to recover Mtb and vague diagnostic criteria. This project will evaluate novel approaches to the diagnosis of AIDS-related pediatric TB in a hyperendemic setting using 1) rapid, cost-effective Mtb culture and susceptibility methods based on direct microscopic observation techniques and 2) alternative non-invasive specimens such as nasopharyngeal aspirates (NPA) and stool to detect Mtb. An optional component will assess improved rapid detection of Mtb by a semi-nested polymerase chain reaction assay (N2 PCR), a technique appropriate for regional reference laboratories in developing countries. Our preliminary data show a high correlation between culture results and N2 PCR results in adults (sputum PCR) and children (stool and NPA PCR) with tuberculosis, and mean time to detection of Mtb by our microscopic observation method was 9 days (at a fraction of the cost of rapid methods used in the U.S.). This is a collaborative effort between PRISMA, a Peruvian private voluntary organization, two U.S. universities (Tulane and Johns Hopkins), and a Peruvian university (Cayetano Heredia). Two hundred-sixty children with pulmonary disease meeting clinical criteria for TB disease (including at least 100 HIV-infected) from the Hospital del Niño, Lima, Peru, and 260 age-matched controls from both high- and low-risk communities will be enrolled. Mtb will be detected in gastric aspirates (cases only), NPAs, and stool by new and traditional culture methods and by N2 PCR. Children with a positive N2 PCR but without clinical evidence of TB requiring antituberculous therapy will be followed longitudinally. These new diagnostic methods have tremendous potential to improve and simplify the diagnosis of pediatric tuberculosis in low-income countries with limited laboratory resources.

Grant: 1R01AI049151-01A1
Program Director: LAUGHON, BARBARA E.
Principal Investigator: CRICK, DEAN C PHD
Title: Sterol/Hopanoid Biosynthesis: An Anti-TB Drug Target
Institution: COLORADO STATE UNIVERSITY-FORT FORT COLLINS, CO
COLLINS
Project Period: 2001/06/15-2005/05/31

DESCRIPTION (provided by applicant): Multi-drug resistant tuberculosis is increasing in prevalence worldwide; therefore, a greater understanding of the basic biochemistry of *Mycobacterium tuberculosis* is of utmost importance. Analysis of the *M. tuberculosis* genome suggests that there may be a biosynthetic pathway analogous to eukaryotic sterol synthesis in this organism. Preliminary evidence indicates that the *M. tuberculosis* genome encodes enzymes with structural homology to several eukaryotic sterol synthesis enzymes including farnesyl diphosphate synthase, squalene synthase, squalene epoxidase, oxidosqualene cyclase and lanosterol 14a-demethylase. It has been shown that both the *M. tuberculosis* farnesyl diphosphate synthase and lanosterol 14a-demethylase are functional as well as structural homologs of the eukaryotic enzymes. More importantly, commercial anti-fungal drugs that are known inhibitors of sterol synthesis (specifically oxidosqualene cyclase and lanosterol 14a-demethylase) effectively inhibit the growth of *M. tuberculosis* in culture. It is hypothesized that *M. tuberculosis* synthesizes cyclic isoprenoid compounds, perhaps sterols or hopanoids, which are essential to the viability of the organism. Therefore, the specific aims of this proposal are to: 1) identify and characterize cyclic isoprenoid compounds in *M. tuberculosis*. 2) isolate, enzymatically characterize and determine the essentiality of the sterol synthesis homologs expressed by *M. tuberculosis*. 3) identify and characterize the active site of the oxidosqualene cyclase homolog. The identification of a sterol/hopanoid biosynthetic pathway in *M. tuberculosis* and characterization of relevant enzymes represents a novel approach to the identification of previously unsuspected antituberculosis drug targets.

Grant: 1R01AI049192-01
Program Director: KLEIN, DAVID L
Principal Investigator: SNAPPER, CLIFFORD M MD
Title: DENDRITIC AND T CELLS IN ANTI-BACTERIAL Ig RESPONSES
Institution: HENRY M. JACKSON FDN FOR THE ADV ROCKVILLE, MD
MIL/MED
Project Period: 2001/04/01-2006/03/31

DESCRIPTION (provided by applicant): Infections due to extracellular bacteria continue to pose a significant global health problem. This is due in large part to the continual emergence of antibiotic-resistant strains. Hence, there exists an urgent need for development of protective vaccines. Immunity is mediated by antibodies to the bacterial polysaccharides (PS) as well as proteins. However, little is known regarding the parameters that mediate in vivo anti-PS and anti-protein responses to intact extracellular bacteria, although such information has relevance to the rational design of immunotherapies for these agents. We have established an in vivo model system for investigating the mechanism of induction of anti-PS and anti-protein Ig isotypes in response to intact *Streptococcus pneumoniae*. Specifically, the Ig isotype response to the phosphorylcholine (PC) determinant, present on the bacterial cell wall C-PS is studied and compared to the humoral response to a cell wall protein, pneumococcal surface protein A (PspA). We show that induction of optimal anti-PC and anti-PspA responses both require CD4⁺TCR- α/β ⁺ T cells and B7-dependent costimulation, although memory fails to develop for induction of PC-specific Ig. Of interest, the mechanisms underlying the T cell-dependence of these two responses are distinct. We further show that dendritic cells (DCs) can phagocytose *S. pneumoniae* upon transfer into naive mice, induce both anti-PC and anti-PspA Ig responses, and the formation of PspA-specific memory. The general aims of this application are to elucidate the mechanisms by which DCs respond to and process an intact extracellular bacterium for induction of both T cell-dependent PC- and PspA-specific Ig isotypes in vivo, and determine the mechanisms underlying the distinct forms of T cell help that stimulate these respective antigen-specific Ig isotype responses. Specifically, we will utilize a number of in vitro and in vivo model systems to determine 1) the parameters that regulate DC activation and antigen presentation in response to R36A, 2) the relative contribution of DC subsets, and 3) the role of DC cytokines and accessory molecules, including CD40, MHC class II, and Toll-like receptors. In this context, 4) the differential requirements for DC stimulation of T cell help for the anti-PC versus the anti-PspA response will be determined. These data will be the first to establish the detailed parameters that mediate a physiological antigen-specific humoral immune response to an intact extracellular bacterium, including the delineation of the fundamental differences between polysaccharide and protein-specific Ig isotype responses.

Grant: 1R01AI049200-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: FIKRIG, EROL MD MEDICINE
Title: Tissue Specific Borrelia Gene Expression
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 2001/05/01-2006/03/31

DESCRIPTION (provided by the applicant): This project seeks to understand how microbial antigens that are selectively expressed in vivo, and in specific tissues, contribute to the genesis of protective host immune responses and can serve as rational targets for vaccines. *Borrelia burgdorferi* has been shown to preferentially express different genes during infection of the mammalian host and within distinct host tissues. Moreover, *B. burgdorferi* has a tropism for the skin, joints, heart and nervous system, and these organs/tissues are associated with particular clinical manifestations of Lyme disease, such as erythema migrans or Lyme arthritis. *B. burgdorferi* genes that are selectively expressed in vivo and in precise host tissues will be identified using 2 strategies. First, differential immunoscreening, a technique developed in our laboratory, will be used. In this approach, a *B. burgdorferi* expression library is probed with 2 sets of sera (for example, [a] sera from an infected host, and [b] sera from a host hyperimmunized with killed *B. burgdorferi*) to identify antigens that only react with [a], and may therefore be selectively expressed in vivo. Secondly, DECAL (Differential Expression analysis using a Custom Amplified Library), will be adapted to identify *B. burgdorferi* genes expressed in the host. In this strategy, prokaryotic ribosomal RNA is removed for a bacterial expression library, which can then be used for subtractive analysis of gene expression in host tissues. Then the immune responses to the host-specific *B. burgdorferi* antigens will be characterized. We will directly assess tissue-specific gene expression, by RT-PCR using patient specimens and also in an experimental murine model of Lyme borreliosis. Then we will correlate antigen-specific immune responses with the course of Lyme disease in patients and determine whether immune responses to tissue-specific antigens influence the course of infection and/or prevent specific clinical manifestations of disease.

Grant: 1R01AI049252-01
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: NORRIS, STEVEN J PHD
Title: Physiologic Characteristics of Treponema Pallidum
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR houston, TX
HOUSTON
Project Period: 2001/05/01-2005/04/30

DESCRIPTION: (Provided by applicant): The complete sequence of the Treponema pallidum subsp. pallidum genome was determined in 1998 providing a new window to the physiology of this enigmatic organism. Availability of the annotated sequence represents a major advance in syphilis research. However, sequence information can only serve to predict function, and functional analysis is a necessary step in the practical application of this data to syphilis prevention and control. This application represents a reactivation of a project aimed at determining the nutritional and environmental requirements of T. pallidum and applying this information toward the in vitro culture and improve understanding of the pathogenesis of syphilis. Specific Aim 1 will focus on the metabolic pathways predicted by the sequence and the application of this information to the in vitro culture of T. pallidum. Both cell-free and tissue-culture systems will be utilized to assess the effects of medium components on T. pallidum survival, multiplication, and DNA replication and damage. Specific Aim 2 will address the relationship between T. pallidum and oxygen, which is key to its survival and growth. The central hypothesis addressed in this Aim is that NADH oxidase and the multimeric protein AhpC play a major role both in maintaining a proper red-ox environment in the cell and in removing reactive oxygen intermediates. In Specific Aim 3, Dr. Milton Saier and colleagues at the University of California at San Diego will examine the role of the phosphoenolpyruvate dependent phosphotransferase system (PTS) in gene regulation. A unique aspect of the T. pallidum genome is that it encodes HPr (PtsH), the HPr(ser) kinase (PtsK), a frameshifted Enzyme I (PtsI) and two additional potential regulatory PTS proteins, but no recognizable sugar-specific PTS permeases. The likelihood that the existing PTS components are involved in a regulatory network independent of transport function will be investigated. It is anticipated that the results of this study will improve understanding of the growth requirements, oxygen utilization, and regulatory systems of T. pallidum.

Grant: 1R01AI049313-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: MOODY, DAVID B MD
Title: T cell response to CDI-restricted lipids in tuberculosis
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 2001/03/01-2006/02/28

DESCRIPTION (provided by the applicant): Mycobacterium tuberculosis has infected one third of all humans, resulting in 2-3 million deaths annually and increasing rates of coinfection with the human immunodeficiency virus (HIV). The main impediment to eradication of tuberculosis relates to the ability of M. tuberculosis to persist long term intracellularly within host tissues. Cellular immune responses, particularly activation of Th1 T cells, are crucial for killing intracellular mycobacteria and successfully resolving tuberculosis infections. Although most studies have evaluated mycobacterial protein antigen for activation of T cells, it is now known that group 1 CD1 molecules (CD1a, CD1b, CD1c) mediate T cell activation by mycobacterial glycolipids, including two classes of glycolipid antigen discovered by the applicant's group, glucose monomycolate (GMM) and mannosyl phosphodolichol (MPD). Preliminary studies indicate that CD1-restricted T cells that recognize MPD and GMM are detectable in the peripheral blood of human subjects infected with M. tuberculosis, but not naive controls, indicating that M tuberculosis infection generates glycolipid-specific T cell responses in vivo. T cells use clonally variable T cell receptors to specifically recognize several mycobacterial glycolipids without crossreactivity. T cell recognition of mycobacterial glycolipids is generally specific for the carbohydrate structure of the antigens, including a product of glycosylation reactions that is produced during intracellular growth within host tissues. Now the applicant proposes to measure polyclonal T cell responses from naive and M tuberculosis infected humans to the major classes of purified mycobacterial glycolipids typical of extracellular and intracellular growth. We will use purified CD1-presented antigens as well as mycobacterial glycolipids that are specifically upregulated during intracellular growth to measure human lymphocyte responses during the first year after infection. Antigen-specific lymphocytes will be detected using proliferation assays, antibody-capture cytokine ELISA (elispot) and glycolipid-loaded CD1 - tetramers. Glycolipid-specific T cells will be characterized with regard to restriction by CD1 - isoforms, dependence on prior infection and expression of cell surface markers of immunological memory. These studies will determine whether human infection by M tuberculosis generally results in acquired T cell responses that are specific for mycobacterial glycolipids expressed during intracellular growth. Determination of the immunodominant glycolipid targets of the human T cell response during natural tuberculosis infection will provide crucial information for development of CD1-presented glycolipids as immunomodulatory agents, including vaccines.

Grant: 1R01AI049418-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: SMALL, PAMELA L. PHD
MICROBIOLOGY:IMMUNOLOGY
Title: Mycolactone-Mediated Virulence in *M. ulcerans*
Institution: UNIVERSITY OF TENNESSEE KNOXVILLE KNOXVILLE, TN
Project Period: 2001/03/15-2006/02/28

DESCRIPTION (provided by the applicant): *Mycobacterium ulcerans* is the causative agent of Buruli ulcer, a severe persistent skin infection which has been recently designated an emerging infection in West Africa. The disease has a unique pathology. Despite extensive tissue damage and the presence of a heavy bacterial load, there is little acute inflammatory response to the organism. A single Buruli ulcer may cover 15 percent of a person's body surface; the only cure is surgery and skin grafting. A polyketide-derived macrolide toxin designated mycolactone has been identified in *M. ulcerans*. Macrolides are produced as secondary metabolites by soil bacteria and fungi. They have enormous pharmaceutical value as cytostats, immunosuppressants, antifungal agents, antihelminthic agents and antibiotics. Mycolactone is the first macrolide identified from a pathogen. Evidence suggests that mycolactone is responsible for most of the pathology in Buruli ulcer. Mycolactone-mediated phenotypes include cell cycle arrest, immunosuppression and death via apoptosis. Neither the genetics of mycolactone synthesis nor its mechanism of action are known. The goals of this proposal are: 1) to clone and sequence genes for mycolactone biosynthesis, 2) to construct mutants defective in mycolactone production, and 3) to begin characterizing events in the cellular pathways involved in mycolactone mediated cell death and immunosuppression using micro-array gene-expression technology. Results from these studies should have a significant impact on the treatment and prevention of Buruli ulcer as well as provide insight into potential role of polyketides in other mycobacterial diseases such as tuberculosis. In addition, this work may provide useful insight into macrolide cell biology.

Grant: 1R01AI049421-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: KRAMNIK, IGOR MD
Title: Genetic susceptibility to Mycobacterium tuberculosis
Institution: HARVARD UNIVERSITY (SCH OF PUBLIC BOSTON, MA
HLTH)
Project Period: 2001/09/30-2006/06/30

DESCRIPTION (provided by the applicant): It is projected that a total of 225 million new cases of tuberculosis will occur between 1998 and 2030. The outcome of primary infection with Mycobacterium tuberculosis (MTB) varies and, in immunocompetent hosts, imparts only a 10 percent lifetime risk of developing clinical disease. The significant variation in tuberculosis susceptibility among immunocompetent individuals remains unexplained. Differences in the outcome of tuberculosis infection in the setting of similar risk factors support a significant role of the host genetic background in predisposition to progression towards clinical disease. Therefore, identification of genetic factors associated with susceptibility to tuberculosis will have important implications for controlling the disease. We use a mouse experimental model of infection with virulent strain of MTB to characterize genes that are responsible for control of tuberculosis infection in immunocompetent hosts. We have identified and mapped to a 2 cM interval on mouse chromosome 1 a novel locus (sst1) that significantly contributes to control of growth of virulent MTB primarily in the lungs. Observations on its phenotypic expression demonstrate that genetic mechanisms controlling infection with virulent MTB are distinct from those that control an avirulent vaccine strain of M bovis BCG. Having generated a set of sst1-congenic inbred strains of mice, we propose to use them (1) to isolate the sst1-candidate genes by positional cloning; (2) to identify genetic polymorphism responsible for tuberculosis susceptibility; (3) to identify cells and functional pathways affected by the sst1 polymorphism. The proposed project will identify the nature and mechanism of action of the sst1 in mice, which in the future should permit isolation of its human homologue and the analysis of its role in tuberculosis susceptibility in humans. The understanding of genetically determined mechanisms that operate during the course of tuberculosis infection in the lung will provide new insights into the pathogenesis of tuberculosis and suggest improved strategies for treatment and prevention of the disease.

Grant: 1R01AI049448-01
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: MOORE, THOMAS A BS
Title: Gamma Delta T Cells in Anti-Bacterial Host Defense
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2001/04/01-2006/02/28

DESCRIPTION (provided by applicant): *Klebsiella pneumoniae* is a leading cause of community-acquired and nosocomial infections. Moreover, *K. pneumoniae* is the second leading cause of nosocomial gram negative bacteremia. The recent emergence of multi-antibiotic resistant strains of *K. pneumoniae* due to extended-spectrum B-lactamase (ESBL) production is cause for significant clinical concern. Interestingly, antibiotic resistance appears to be more prevalent in blood isolates than from other sources. While the innate host response towards gram-negative bacterial infections has been characterized, little is known about gamma delta-T cells and their role in these infections. Our preliminary studies indicate that gamma delta-T cell knockout (KO) mice have significantly impaired early expression of pulmonary and hepatic IFN-gamma and TNF-alpha mRNA following intratracheal *K. pneumoniae* infection, increased blood bacterial dissemination, and increased hepatic bacterial burden subsequent to the initial pulmonary infection. Additional studies indicate increased mortality following intravenous bacterial inoculation in gamma delta-T cell KO mice and uncontrolled blood bacterial growth. Combined, our preliminary data suggest gamma delta-T cell KO mice succumb from an impaired ability to clear disseminated bacteria rather than from an inability to clear the organism from the primary pulmonary infection. The hypothesis of this proposal is that gamma delta T cells play a critical role in the host acute inflammatory response during gram-negative bacteremia via recognition of heat shock protein 60 expression in the liver following infection. A murine model of blood-borne *K. pneumoniae* infection will be used to perform the following Specific Aims: 1) To contrast the host response in gamma delta T cell knockout and wildtype mice during *K. pneumoniae* bacteremia, 2) To assess the kinetics of gamma delta T cell A) recruitment and activation and B) cytokine production during *K. pneumoniae* bacteremia, 3) To reconstitute resistance to *K. pneumoniae* in gamma delta T cell knockout mice by adoptive transfer of gamma delta T cells from wildtype and cytokine deficient (IFN-gamma or TNF-alpha) mice, 4) To confer resistance to *K. pneumoniae* in gamma delta T cell knockout mice by TNF-alpha or IFN-gamma reconstitution using systemic adenovirus gene therapy, and 5) To assess the requirement of heat shock protein 60 for gamma delta T cell activation during *K. pneumoniae* bacteremia. These studies will provide insights for the development of therapeutic modalities aimed at augmenting host responses, resulting in enhanced resolution of bacterial infections.

Grant: 1R01AI049497-01
Program Director: LAUGHON, BARBARA E.
Principal Investigator: NACY, CAROL A PHD
Title: High throughput TB drug screens
Institution: SEQUELLA GLOBAL TUBERCULOSIS FOUNDATION BETHESDA, MD
Project Period: 2001/09/20-2004/08/31

DESCRIPTION (adapted from applicant's abstract): The search for new TB drugs has finally caught up with the pharmaceutical revolution of the last half of this century: solid-matrix-based methods of chemical synthesis now create combinatorial chemistry libraries of pharmaceuticals that contain millions of unique compounds. Using robotics and sophisticated assay instrumentation, these libraries can be screened with high throughput to identify compounds which are lethal to specific organisms, or which affect specific pathways known to be critical for bacterial survival. A year-old CRADA between the NIAID and Sequella, Inc. to develop combinatorial chemistry and a high throughput screening assay succeeded in synthesizing and screening approximately 100,000 analogues of the TB drug ethambutol in a very short period of time. The gene-based screen used in these studies identified compounds that affect specific regions of the M. tuberculosis genome that are activated in response to ethambutol therapy, and are involved in the cellular response to cell wall damage. The Sequella, Inc./NIH collaboration had impressive momentum (100,000 compounds screened, 200 hits, 4 lead compounds in 9 months). The Foundation intends to provide several gene-based and whole-cell high throughput screening assays to interested pharmaceutical companies to screen their chemical libraries in a similar timeframe. One company, for example, has 500,000 well-characterized chemicals that have never been tested for TB. They are interested in providing the Foundation with these chemicals to test in a specific whole cell-screening assay as an initial screen for hits. Setting up the high throughput screen in-house, while not that expensive, represents an opportunity cost for that company and would require a BL3 facility. In addition, a validated whole-bacteria high throughput screening assay would be of benefit to the current NIAID/DAIDS drug screening program. The Foundation would be interested in transferring the screen to the NIH program over the course of this challenge grant. The Foundation goal is to reduce the barriers to entry that the major pharmaceutical companies face when considering the market for new TB drugs. Thus, providing tailored screening programs that support the specific needs of pharmaceutical partners is a relatively inexpensive endeavor that may improve the likelihood that medicines will emerge to improve the treatment of this neglected disease.

Grant: 1R01AI049577-01
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: FRANK, DARA W
Title: Activity of Pseudomonas Type III Toxins
Institution: MEDICAL COLLEGE OF WISCONSIN MILWAUKEE, WI
Project Period: 2001/06/01-2006/04/30

DESCRIPTION (provided by the applicant): *Pseudomonas aeruginosa* is an opportunistic pathogen that expresses a wide variety of virulence determinants. Our work has focused on the contribution of one extracellular bacterial product to pathogenesis, exoenzyme S. Exoenzyme S is a member of the family of ADP-ribosyltransferase enzymes. Production of exoenzyme S is correlated with the ability of *P. aeruginosa* to spread or disseminate from epithelial colonization sites to the bloodstream of infected individuals, resulting in the development of a fatal sepsis. Our initial models of the intoxication mechanism for ExoS were simplistic and based on the notion that ExoS would exhibit an A:B structure. We subsequently showed, however, that ExoS was delivered into the cytosol of eukaryotic cells by a type III mechanism of intoxication. These observations opened new areas of investigation resulting in the cloning and sequence analysis of the *Pseudomonas* type III system, characterizing the major extracellular proteins secreted by the type III apparatus, and discovering two new toxins, ExoU and ExoY. The long-term goals of this new proposal are to determine the mechanism of action of ExoU and to begin expression and biochemical studies on ExoY. ExoU expression is responsible for the acute cytotoxic response in cultured cells and lung injury in vivo. ExoU possesses no known motifs, enzymatic activity, or homology to other proteins in the data base and likely represents a novel toxin. Structure-function analysis indicates that the cytotoxic response of ExoU is encoded in at least two domains that can function in trans within mammalian cells. New data, presented in this application, demonstrate that ExoU is toxic to yeast. We will use yeast as a model genetic and biochemical system to identify the target of ExoU toxicity. ExoY possesses adenylate cyclase activity and is related to the adenylate cyclase toxins of *Bacillus anthracis* and *Bordetella pertussis*. Although the activity of ExoY is known, the association of ExoY expression and the ExoY activity have not been examined relative to the pathogenesis of *P. aeruginosa*. Understanding the mechanisms of action of ExoU and ExoY will aid in the design of alternative treatments.

Grant: 1R01AI049778-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: SALGAME, PADMINI PHD
Title: Th1 Cell Apoptosis in Tuberculosis
Institution: TEMPLE UNIVERSITY PHILADELPHIA, PA
Project Period: 2001/04/01-2006/03/31

DESCRIPTION: The protective immune response operative in tuberculous infection is not completely defined. Nevertheless, it is generally accepted that Th1-mediated immunity plays a significant role in engendering protection against *M. tuberculosis*. This notion is perhaps best supported by the fact that HIV-infected individuals, in whom Th1 cell apoptosis occurs, are highly susceptible to the tubercle bacillus. Emerging evidence suggests that apoptosis of T cells, including the Th1 subtype, occurs during tuberculous infection. Our laboratory has recently observed that Th1 and Th2 cells can be differentially modulated to undergo apoptosis. We previously reported that CD95-mediated apoptosis in response to CD3/TCR (T cell receptor complex) ligation is observed only in Th1, and not in Th2 T cell clones. In contrast to Th2 lymphocytes, Th1 cells demonstrated a distinct requirement for costimulation via ligation of specific T cell surface components with co-stimulatory molecules of antigen presenting cells such as macrophages. In addition to CD3 signals, to resist CD95-mediated apoptosis. This difference in sensitivity to apoptosis of the two Th subsets is due to selective upregulation of phosphatidylinositol 3'-kinase (PI3'-K) activity in Th2 clones following CD3 ligation. The upregulation of PI3'-K activity abrogates apoptosis by inhibiting CD95 aggregation in the membrane thereby blocking subsequent activation of the death effector molecule, caspase-3. Thus, activation of Th1 cells, in a manner akin to antigen stimulation in the absence of co-stimulation, results in their apoptosis. Importantly, we have observed that expression of the B7 co-stimulatory molecule is down-regulated in *M. tuberculosis*-infected macrophages. Based on these observations, we propose to test the hypotheses that i,) in tuberculous infection, CD95-mediated Th1 depletion occurs, resulting in attenuation of protective immunity against *M. tuberculosis*, thereby enhancing disease susceptibility; ii) downregulation of the expression of the B7 class of costimulatory molecules contributes to Th1 apoptosis. It is hoped that elucidation of the mechanisms underlying CD95-mediated Th1 death in tuberculosis will help develop strategies to enhance protective immunity against the tubercle bacillus via specific disengagement of the apoptosis machinery in Th1 cells.

Grant: 1R01AI049950-01
Program Director: LANG, DENNIS R
Principal Investigator: WAKSMAN, GABRIEL PHD
Title: Structure of Proteins Involved in Bacterial Pathogenesis
Institution: WASHINGTON UNIVERSITY ST LOUIS, MO
Project Period: 2001/06/01-2006/04/30

Bacterial pathogenesis involves at least two steps: 1- attachment of the bacteria to the host tissue to be infected, and 2- secretion of toxic molecules by the bacteria. Both steps are mediated by a fibrous structure displayed at the surface of the bacteria called a "pilus". On one end of the pilus (facing outwards), the pilus harbours a protein called "adhesin" which binds specifically to the host's surface polysaccharides. On the other end, the pilus may be attached to a secretion machinery responsible for injection of toxic substances. The pilus itself is a complex polymer of several different protein subunits. In this proposal, we propose to study the structural basis of 1- pilus biogenesis, 2- bacterial attachment to the host tissue, and 3-protein secretion. We have used the type P pili of uropathogenic *Escherichia coli* as a model to study pilus biogenesis and bacterial attachment, and we have used the type IV secretion system of the ulcer-causing *Helicobacter pylori* as a model to study the secretion of proteins by bacteria. We have obtained several crystals of pilus subunits in complex with their assembly chaperone; we have also crystallized binary complexes of adhesins with their cognate polysaccharides; and finally, we have crystallized important components of the type IV secretion machinery. Two of these structures have been or are in the process of being solved. Our proposal, by seeking to understand the structural basis of pathogenicity in bacteria responsible for important infectious diseases, will have not only an impact on the fundamental knowledge of the various systems under study, but will also help design antibiotic compounds which are effective in the fight against these diseases.

Grant: 1R01AI049973-01
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: SO, MAGDALENE Y PHD
MICROBIOLOGY:BACTERIOLOGY
Title: Formation of Cortical Plaques by Neisseria
Institution: OREGON HEALTH & SCIENCE UNIVERSITY PORTLAND, OR
Project Period: 2001/08/01-2006/05/31

DESCRIPTION (provided by the applicant): The Neisseria type IV pilus modulates bacterial infectivity by mediating adhesion and inducing cell signaling pathways. Upon binding its receptor, CD46, the pilus causes a transient increase in cytosolic free Ca^{2+} levels, triggering endosome and lysosome exocytosis. Piliated bacteria next trigger elongation of microvilli and formation of cortical plaques at the plasma membrane beneath the site of contact. Cortical plaques contain clusters of Opa receptors, transmembrane signaling proteins, actin microfilaments and ezrin, a protein that tethers the membrane to the actin cytoskeleton. These plaques serve multiple signaling functions that promote bacterial infection. Unlike the Ca^{2+} response, which can be induced with purified pili, cortical plaque formation requires live diplococci and PilT, a protein that functions in pilus assembly and DNA transformation. PilT also controls pilus retraction, a process that drives twitching motility and the ability of diplococci to aggregate into microcolonies. Retraction generates substantial force on the substrate to which the pilus is attached. External forces placed on the membranes of eucaryotic cells result in the induction of kinase cascades, cytoskeleton reorganization and alterations in translation. The tension generated on the plasma membrane by retraction of pili during infection may therefore act as a signal to promote the formation of cortical plaques and subsequent bacterial invasion. Based on these and other observations, we propose a model for early events in pilus-induced cortical plaque formation. In this proposal, we describe experiments to test key predictions of this model.

Grant: 1R01AI049976-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: PHILIPP, MARIO T
Title: Lyme disease: A possible test for cure
Institution: TULANE UNIVERSITY OF LOUISIANA COVINGTON, LA
Project Period: 2001/07/01-2004/05/30

DESCRIPTION (provided by applicant): It would be immensely useful for the management of Lyme disease (LD) treatment to have available a test for cure. Such a test could be employed not only to ascertain if treatment of acute LD was successful, thereby preventing the transition to the chronic, more intractable form of the disease, but also to distinguish among the possible etiologies of the so-called post-treatment LD syndrome. The PI and coworkers recently developed a sensitive and specific enzyme-linked immunosorbent assay (ELISA) for the serological diagnosis of LD. The test is based on the detection of antibody (Ab) to an immunodominant, invariable region (IR) of the lipoprotein VIsE. VIsE is the molecule that undergoes antigenic variation in *Borrelia burgdorferi* (the etiologic agent of LD). A peptide (C6) representing the invariable region 6 (IR6) of VIsE serves as antigen. It is hypothesized that, because the spirochete should not simultaneously express on its surface more than one (or a few) VIsE variant(s) at any time, the VIsE lipoprotein must be rapidly turned over and degraded by the spirochete as new variants are progressively expressed. As a consequence of this postulated intrinsic instability, VIsE should be scarce on dead or dying spirochetes, and secondary Ab responses to the C6 peptide should decline in unison with the infection's demise, following antibiotic treatment. It is further hypothesized that the decline in titer of the C6 Ab as a function of time after treatment may serve as a test for Lyme disease cure. Preliminary results indicate that the C6 ELISA titer in cured patients falls by a factor greater or equal than 4 whereas for treatment-resistant patients the fall is by a factor <4 . This is similar to the VDRL test used to diagnose syphilis cure. The broad, long-term objective of this project is to assess both retrospectively and prospectively the ability of the C6 ELISA to serve as a test for LD cure. In this proposal the C6 test will be assessed retrospectively by achieving three specific aims: Specific Aim 1: To assess retrospectively the C6 ELISA as a test for cure in patients with acute LD. Serial serum samples from patients with either erythema migrans (n = 90) and/or culture-confirmed infection (n = 156) will have been collected at presentation and at 6 and 12 months thereafter. The samples will be titrated for anti-C6 Ab. Specific Aim 2: To assess retrospectively the C6 ELISA as a test for cure in patients with chronic LD and post-treatment LD syndrome. Same as for SA1, but with patients with chronic LD (n = 150) and post-treatment LD syndrome (n = 60). Specific Aim 3: To assess the C6 ELISA as a test for cure in animal models of LD. Cure of LD will be assessed objectively (by culture and PCR) both in rhesus monkeys (chronic LD) and in mice (acute LD). Correlation between LD cure and anti-C6 Ab titers will be evaluated.

Grant: 1R01AI050002-01
Program Director: LANG, DENNIS R
Principal Investigator: AHMER, BRIAN M BS
Title: Detection of Mixed Microbial Communities by Salmonella
Institution: OHIO STATE UNIVERSITY COLUMBUS, OH
Project Period: 2001/08/01-2005/05/31

Despite decades of research on *Escherichia coli* and *Salmonella typhimurium*, microbiologists are still unable to assign, or even convincingly predict, functions for more than 30 percent of the open reading frames (ORFs) in the *E. coli* genome sequence. It seems likely that the functions of many of these genes may not be observable using pure cultures. In nature, such bacteria do not normally exist as pure cultures and a percentage of their genetic capacity is almost certainly involved with 'mixed community' interactions. Consistent with this hypothesis, we recently identified an *S. typhimurium* receptor that is activated only within a mixed microbial community. The signaling event discovered is the detection of N-acylhomoserine lactones (AHLs) by SdiA, a LuxR homolog found in *S. typhimurium*. The *sdiA* gene is also present in *E. coli* and *Klebsiella* spp, suggesting that it has a relatively ancient function. However, what is truly remarkable is that these species do not produce the ligands that are detected by SdiA. Instead, SdiA detects uncharacterized compounds present in mammalian intestines and AHLs produced by other species of bacteria. We will test the hypothesis that the intestinal compounds are microbial in origin and attempt to identify the species producing these molecules. We will use microarrays to identify the portions of the *E. coli* and *S. typhimurium* genomes that are regulated by SdiA in response to AHLs and characterize the phenotypic consequences of this signaling event.

Grant: 1R01AI050022-01
Program Director: KLEIN, DAVID L
Principal Investigator: CARBONETTI, NICHOLAS H PHD
Title: Pertussis Toxin Trafficking and Processing in Cells
Institution: UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD
SCHOOL
Project Period: 2001/07/01-2006/05/31

DESCRIPTION (provided by the applicant): The short-term goal of this project is to understand the trafficking and activation of pertussis toxin (PT) within mammalian cells. *Bordetella pertussis* colonizes the human respiratory tract and causes the disease pertussis (whooping cough). Several systemic symptoms accompany this disease, even though *B. pertussis* is not thought to invade beyond the respiratory tract. Instead these symptoms are thought to be due to the action of PT, an exotoxin produced by *B. pertussis*. PT is an ADP ribosyltransferase that modifies several heterotrimeric G proteins, causing a wide range of effects on signaling in mammalian cells. How PT is transported within mammalian cells to arrive at its target proteins in an active form is largely unknown. In addition, how PT is transported from the respiratory tract to systemic sites is completely unknown. Understanding the mechanisms utilized by this complex toxin to achieve these effects will provide key information on the cell biology of PT and will help to provide a groundwork for studies to elucidate the role of this toxin in *B. pertussis* infection and disease. In addition, this information may allow development of therapeutics to combat the effects of the toxin and may also allow improvement of existing pertussis vaccines that include PT or development of novel vaccine molecules using PT as an intracellular delivery vector. We have preliminary data indicating that (i) PT may undergo retrograde intracellular trafficking through the Golgi apparatus and endoplasmic reticulum (ER) en route to its cytosolic target proteins, (ii) that proteolytic processing of the active Si subunit of cell-associated PT occurs and may be important for its activity, and (iii) that there is apparent transcytosis of active PT across intact polarized epithelial cells in culture. Therefore the specific aims of this proposal are to investigate the trafficking, processing and transcytosis of PT in mammalian cells and the key features of this toxin that mediate these events.

Grant: 1R01AI050230-01
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: GOLDBERG, JOANNA B
Title: Synthesis of P.Aeruginosa LPS and its Role in Infection
Institution: UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA
CHARLOTTESVILLE
Project Period: 2001/09/17-2005/07/31

DESCRIPTION (provided by the applicant): *Pseudomonas aeruginosa* is an opportunistic pathogen that can infect many different tissues. Similar to what has been observed in other Gram-negative bacteria, one of the virulence factors important for acute *P. aeruginosa* infections is lipopolysaccharide (LPS). In the case of *P. aeruginosa*, the O antigen portion is thought to be essential for systemic infections. Chronic lung infections in cystic fibrosis patients, on the other hand, are caused by *P. aeruginosa* strains that have a defective LPS with few or no O antigens; these infections remain localized to the lung. In other *P. aeruginosa* respiratory infections dissemination to the blood is also rare, suggesting that O antigen expression, which confers resistance to the action of normal human serum, may not be required for infections localized to the lung. Prior evaluations of *P. aeruginosa* LPS mutants in various infection models used either genetically or structurally undefined strains, or those with pleiotropic effects. Therefore the question as to the role of O antigen in *P. aeruginosa* pathogenesis clearly needs to be reevaluated. The objective of this proposal is to determine which portions of the LPS are critical for infections at particular sites. The genetic locus encoding the enzymes for the synthesis of *P. aeruginosa* serogroup O11 O antigen has been cloned from strain PA103. Previous work from a number of laboratories has shown PA103 to be highly virulent in many animal models of infection. The steps in the pathway of synthesis of this O antigen will be characterized through mutational, structural, and biochemical analysis of the genes of the O antigen locus (Specific Aim 1). The transcriptional organization of the O antigen gene locus will also be determined (Specific Aim 2). In order to ascertain which portions of the *P. aeruginosa* LPS are required for virulence, the genetically and structurally defined LPS mutants constructed here will be tested in two different mouse models of infection. In the intranasal infection model, infections can remain localized to the lung or can translocate from the lungs to the blood and other organs. In the corneal infection model, the *P. aeruginosa* infection remains localized to the eye after trauma (Specific Aim 3). The long-term goal of this research is to devise rational strategies to target LPS synthetic enzymes for the development of vaccines and antibacterial agents to inhibit infections caused by this important human pathogen.

Grant: 1R01AI050552-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: PLANO, GREGORY V PHD
Title: Structure of the Yersinia pestis type III export complex
Institution: UNIVERSITY OF MIAMI-MEDICAL CORAL GABLES, FL
Project Period: 2001/09/01-2005/08/31

DESCRIPTION (provided by applicant): The use of aerosolized Yersinia pestis as a biological weapon could potentially produce massive numbers of casualties. Weaponized plague could be rendered resistant to most antibiotics and delivered via an aerosol. Thus, in order to protect the military and civilian populations from both endemic and recombinant Y. pestis, new biological countermeasures must be developed that target essential pathogen vulnerabilities. The Y. pestis type III secretion (TTS) system enables the bacterium to subvert or destroy eukaryotic cells via the delivery of anti-host effector proteins. Disruption of the TTS process renders Y. pestis avirulent, indicating that the TTS process represents an attractive target for therapeutic intervention. Assembly of the TTS apparatus requires the participation of at least 21 Ysc (Yersinia secretion) proteins, Ysc proteins are hypothesized to assemble into a supramolecular structure that functions as a protein secretion and injection device. We propose to investigate the assembly and structure of the TTS apparatus. In Specific Aim 1, experiments are described to identify the protein interactions involved in the assembly of the TTS complex. Immunoprecipitation methodologies and yeast two- and three- hybrid studies will identify Ysc proteins that directly interact with one another. The overall objective of Specific Aim 2 is to establish conditions for the solubilization, purification and visualization of the assembled or partially assembled TTS complex. Environmental scanning electron microscopy (ESEM) of fixed samples and/or transmission electron microscopy (TEM) of negatively-stained samples will be used to visualize the surface-exposed and/or isolated TTS complex and/or partially assembled intermediates of this complex. In Specific Aim 3, experiments designed to identify and characterize chromosomally-encoded proteins that are required for the assembly and function of the TTS complex are described. Completion of these Specific Aims will not only provide essential information about the assembly and function of the Y. pestis TTS complex, but may also facilitate the development and/or design of novel therapeutics that target this essential virulence system.

Grant: 1R01AI050553-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: TSOLIS, RENEE M PHD
Title: Characterization of the Brucella abortus virB locus
Institution: TEXAS A&M UNIVERSITY HEALTH SCIENCE COLLEGE STATION, TX
CTR
Project Period: 2001/07/01-2005/06/30

DESCRIPTION (provided by applicant): Brucella abortus is a facultative intracellular pathogen that is highly infectious by the aerosol route and causes chronic, debilitating disease. A key step in B. abortus infection is the establishment of persistent infection within macrophages. The bacterial genes encoding virulence mechanisms required for specific interactions between Brucella and the macrophage remain largely undiscovered. We have identified a genetic locus of B. abortus, virB, that is required for establishing infection both in macrophages and in the mouse model. The B. abortus virB locus is predicted by sequence homology to encode a type IV secretion system. Our long-range goal is to elucidate the mechanism by which the virB locus mediates intracellular survival and persistent infection. The objective of this application is to study the expression of the virB genes and compare the interaction of wild type B. abortus and virB mutants with regard to vacuolar trafficking in the macrophage. The central hypothesis of this application is that the virB locus mediates a critical interaction with the macrophage that allows B. abortus to establish infection. The rationale for the proposed research is that characterization of B. abortus virulence factors mediating specific interactions with macrophages will form the basis for new approaches to treat or prevent brucellosis. We are uniquely prepared to undertake the proposed research, because we have generated tools for studying virB expression at both the transcriptional and translational level. Furthermore, the work will be performed in an excellent research environment that is conducive to its completion. Our Department contains several funded investigators working on intracellular bacterial pathogens and excellent BL-3 facilities, as well as other shared resources available for the study of host/pathogen interactions. The central hypothesis will be tested, and the objectives of this application accomplished by pursuing the following two specific aims: (1) Identify conditions for In vitro and in vivo expression of the B. abortus virB locus and localize protein products in the bacterium, and (2) Determine the mechanism by which the virB locus enables B. abortus to survive and grow intracellularly within macrophages. We expect that the results of this work will provide the first direct evidence for expression of the B. abortus virB proteins as well as define the environmental signals that induce expression of this locus. Furthermore, our results will provide information essential to defining the cellular interaction mediated by the virB locus. These results will be significant, because they are expected to provide new targets for preventive or therapeutic interventions to be employed in the case of illegitimate use of this bacterial pathogen. In addition, it is expected that these results will advance our knowledge of type IV secretion systems, which are used by a number of different bacterial pathogens to subvert the host's defense mechanisms.

Grant: 1R01AI050557-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: RADULOVIC, SUZANA PHD
Title: Molecularly Altered Rickettsiae and Vaccine Development
Institution: UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD
SCHOOL
Project Period: 2001/07/01-2006/05/31

DESCRIPTION (provided by applicant): Despite the worldwide distribution of rickettsial diseases and the highly pathogenic nature of Rickettsiae, there is a substantial gap in our knowledge about their mechanisms of pathogenesis. Although rickettsiae offer a fascinating model of intracellular parasitism, there are significant problems associated with studying an obligate intracellular parasite. The rickettsiae are not amenable to sophisticated studies due to a lack of genetic tools, which has resulted in slow progress in correlating rickettsial genes and gene function. The long-term goal of this proposal is to select and characterize *R. prowazekii* genes that contribute to virulence and pathogenesis, and to identify one or more gene products as candidate targets for vaccine against typhus group rickettsiae. This goal will be achieved via selection, cloning and the expression of genes encoding *R. prowazekii* virulence-associated proteins, and their use in immunoprotection against typhus group rickettsiae (*R. prowazekii* and *R. typhi*). Experiments will be performed to identify and characterize *R. prowazekii* genes encoding proteins for specific use in immunoprotection against typhus group rickettsiae (*R. typhi* and *R. prowazekii*). Rickettsial homologs of the genes involved in cell division, adhesion, and invasion of host cells will be selected from *R. prowazekii* genome sequence, and gene expression studies as well as functional analyses will be carried out. In addition, we will generate *R. prowazekii* and *R. typhi* mutants lacking functional targeted genes. These mutants will be used for functional analysis and their inclusion in developing attenuated non-virulent strains for a broad-based protective vaccine against pathogenic rickettsiae.

Grant: 1R01AI050564-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: KLOSE, KARL E
Title: An Oral Vaccine Against Multiple Biowarfare Agents
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR SAN SAN ANTONIO, TX
ANT
Project Period: 2001/08/01-2005/07/31

DESCRIPTION (provided by applicant): Background: U.S. citizens, particularly military personnel, are vulnerable to the threat of exposure to biological warfare agents. Two such bacterial agents, *Bacillus anthracis* and *Francisella tularensis*, can be easily spread by aerosolization causing a high level of mortality, and are therefore considered to be candidate warfare agents. New vaccines against these and other potential warfare agents are needed which can be easily administered and provide high levels of protection against aerosolized bio-weapons. We have developed a *Salmonella typhimurium* strain (delta-glnA delta-glnH) with a number of attributes that make it an attractive candidate for a live attenuated multivalent vaccine. Our hypothesis is that this attenuated *S. typhimurium* strain can be used as a single oral vaccine to deliver multivalent antigens and provide both mucosal and systemic protective immunity against aerosolized biological warfare agents, specifically *B. anthracis* and *F. tularensis*. We will exploit specific *S. typhimurium* promoters (e.g., *pmrH*) to drive high-level expression of *B. anthracis* and *F. tularensis* antigens within the lymphoid tissue, and thus generate a sufficient immune response with a single dose. The Specific Aims of this project entail: (1) Construction of delta-glnA delta-glnH attenuated *S. typhimurium* vaccine strains with the *pmrH* promoter driving expression of *B. anthracis* Protective Antigen (PA) and *F. tularensis* FopA and TUL4 proteins; (2) Evaluation of the efficacy of vaccine strains (Specific Aim 1) to express heterologous antigens within immune tissue and elicit an appropriate immune response; and (3) Challenge vaccinated animals with aerosolized *B. anthracis* and *F. tularensis* to determine efficacy of the vaccine strains. Our Study Design incorporates collaborative vaccine development at three different sites in San Antonio, based upon the expertise found at each site. The *S. typhimurium* vaccine strains expressing *B. anthracis* and *F. tularensis* antigens will be constructed and inoculated into animals in the laboratories of two *S. typhimurium* researchers, Drs. Karl Klose and John Gunn, at the University of Texas Health Science Center. The evaluation of levels of antigen expression within immune tissue will be carried out at the Brooks Air Force Base by Dr. Kenton Lohman. Aerosolized *B. anthracis* and *F. tularensis* challenge studies of vaccinated animals will take place in the Biosafety Level 4 (BSL-4) laboratory at the Southwest Foundation for Biomedical Research under the guidance of Dr. Jean Patterson. We will be taking advantage of this high-level biocontainment laboratory to perform the aerosol challenges necessary to prove the efficacy of this vaccine approach. Relevance: The development of a single oral vaccine that can simultaneously provide protection against multiple bio-warfare agents would be of tremendous benefit to the health of military personnel and other citizens exposed to these agents. If this vaccine strategy proves successful, additional antigens can be expressed from the same vaccine strain, offering an adaptive and protective health tool.

Includes RPGs (Research Project Grants)
Excludes Clinical Trials

Grant: 1R01AI050565-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: NIERMAN, WILLIAM C PHD
Title: Microarray Expression Analysis of Burkholderia Mallei
Institution: INSTITUTE FOR GENOMIC RESEARCH ROCKVILLE, MD
Project Period: 2001/09/30-2004/08/31

DESCRIPTION (provided by applicant): Burkholderia mallei, one of the organisms used by the Soviets in their bioweapons program, possesses properties that contribute to its potential as a biological weapon. The disease, glanders, can result from an extremely low infectious dose inoculated by either aerosol, oral, or parental routes. The incubation period is short and no effective vaccine is available. Although glanders is a serious life-threatening zoonotic disease, relatively little is known about the pathogenesis, virulence factors, strain differences, and the host immunopathologic responses to infection. The sequencing of the complete genome of this organism is now underway at The Institute For Genome Research (TIGR). This proposal will constitute an initial effort to use the genome sequence of the organism in studies of its pathogenicity. A whole genome glass slide microarray will be constructed from PCR products representing each ORF in the genome. This microarray will be used in a series of experiments to explore the genetic response of this organism to infection of a model animal, the Syrian hamster. Comparative genome hybridization will be conducted with genomic DNA from a closely related non- pathogenic Burkholderia strain, Burkholderia thailandensis, using the microarray to further identify candidate virulence determinants. Genes involved in the establishment of a successful infection and evasion of the host immune system will be identified as will vaccine and drug target candidates. Function and virulence validation of the identified virulence genes will be explored by construction and phenotype analysis of targeted knockout strains.

Grant: 1R01AI050566-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: TURNBOUGH, CHARLES L PHD
Title: CHARACTERIZATION OF B ANTHRACIS EXOSPORIUM PROTEINS
Institution: UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL
BIRMINGHAM
Project Period: 2001/09/01-2005/08/31

DESCRIPTION (provided by applicant): In recent years a growing number of foreign governments and radical groups, many unfriendly to the United States and its allies, have added *Bacillus anthracis*, the cause of anthrax, to their arsenals of weapons of mass destruction. This bacterium is convenient for use in warfare and terrorism because it is highly pathogenic, can be grown easily, and forms spores. The spore's resistance to extreme temperatures, noxious chemicals, desiccation, and physical damage make it suitable for incorporation into explosive weapons and for concealment in terrorist devices. Spores can enter the body through ingestion or by inhalation, germinate into vegetative cells, and cause death within 1 to 7 days, sometimes with little or no overt sign of infection preceding death. Antibiotics can be used to treat anthrax, but *B. anthracis* strains have been, or can be, constructed that resist these drugs. A vaccine against an anthrax toxin exists, but it is slow acting and difficult to produce. Thus, better responses to the threat of anthrax are needed. The overall goal of this proposal is to identify protein factors on the surface of the *B. anthracis* spore that contribute to pathogenesis and are potential targets for new vaccines and drug intervention. The outermost layer of the *B. anthracis* spore is a semi-permeable, loose-fitting, balloon-like structure called the exosporium. Approximately 50 percent of the exosporium is composed of a protein consisting of perhaps eight unique protein components. At present, no integral exosporium proteins of *B. anthracis* have been identified. However, growing evidence suggests that they will contribute significantly to key spore properties such as structure, viability, germination, infectivity, and virulence. In the proposed research, we will identify the proteins in the *B. anthracis* exosporium and attempt to assign a function to each. The three specific aims of this proposal are: (1) Identify the proteins present in the exosporium of *B. anthracis* and the genes that encode these proteins. This will be accomplished by isolating proteins, determining partial sequence by mass spectrometry, and identifying proteins and their genes with the aid of the sequenced *B. anthracis* genome; (2) Mutationally inactivate the genes encoding the exosporium proteins and examine the effects on the key spore properties listed above; and (3) Use an existing panel of monoclonal antibodies against the *B. anthracis* spore surface (i.e., exosporium) to create affinity-matured single chain antibodies that neutralize exosporium protein activity, then test the effects of these reagents on spore properties.

Grant: 1R01AI050576-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: POPOV, SERGUEI G PHD
Title: Role of hemolysins in escape of anthrax from macrophages
Institution: ADVANCED BIOSYSTEMS, INC. ALEXANDRIA, VA
Project Period: 2001/09/30-2004/07/31

DESCRIPTION (provided by applicant): This proposal studies the role of hemolysins as virulence factors for *Bacillus anthracis*. Hemolysins allow certain virulent bacterial species to escape the phagosome of macrophages. Using the sequence of *Listeria monocytogenes* hemolysins for comparison, the incomplete genome of *B. anthracis* was screened for the presence of hemolysin genes. Five open reading frames containing protein-coding sequences homologous to *Listeria* hemolysins or associated proteins were discovered in the *B. anthracis* chromosome. These hemolytic proteins, named anthralysins 0, A, B (AnLO, AnLA, AnLB); p3058, and p3201, have not been previously characterized. The goals of this proposal are to: (1) Characterize these hemolytic genes by studying gene expression patterns and regulation; (2) Address the role of each individual anthralysin gene in anthrax pathogenesis; and (3) Study means to inhibit the activity of anthralysins. Such understanding could lead to the development of new antibiotic compounds which act by inhibiting hemolytic proteins and preventing release of anthrax bacilli from the macrophage phagosome, preventing anthrax-associated macrophage death and blocking the infection before it can become systemic.

Grant: 1R01AI051667-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: COX, JEFFERY S BS
Title: Role of MmpL transporters in M. tuberculosis virulence
Institution: UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA
Project Period: 2001/09/15-2006/08/31

DESCRIPTION (provided by applicant) Tuberculosis (TB) is a persistent lung infection that has plagued mankind for centuries and ranks as one of the most serious threats to world health today. The 2-3 million deaths attributed yearly to the disease, as well as the emergence of strains resistant to all of the available chemotherapeutic agents, urgently call for the development of new therapies to treat TB. For years, the identification of new drug targets has been hampered by the intractability of the bacillus to genetic analysis. Now with the advent of powerful genetic tools, combined with well-established mouse infection models, we have isolated novel M. tuberculosis mutants with lesions in individual genes that are required for normal growth during acute infection. Our initial results have led us to the hypothesis that M. tuberculosis influences host-pathogen contacts by utilizing the MmpL family of transporters to secrete biologically active lipids to the surface of the mycobacterial cell and ultimately into infected host cells. The studies proposed here give us the opportunity to test this model and thus understand the molecular details host-pathogen interactions critical during this stage of M. tuberculosis infection. Specifically, we will study a subset of Mmpls that are required for disease and identify the host-pathogen interactions mediated by these virulence molecules. We will determine the mechanism of transport of the cell wall lipid phthiocerol dimycocerosate (PDIM) by MmpL7 and seek to understand why this molecule is important for lung specificity of M. tuberculosis. Furthermore, we will identify the molecules transported by the other MmpL proteins identified by our genetic screens and determine their role in pathogenesis. Finally, we will determine if these molecules serve distinct roles in modifying the host for the benefit of the bacterium. Because members of the MmpL family of transporters are highly homologous to one another and to MmpL proteins of other mycobacterial pathogens, understanding the common mechanisms of their function may lead to the development of inhibitors that could be useful for treating a broad range of infectious diseases. The results from these studies will direct our long-term plans to understand the role secreted lipids play in the struggle between M. tuberculosis and the host. Ultimately, by understanding tuberculosis pathogenesis at the molecular level, we hope to aid in the discovery of new therapies to combat and eradicate this persistent infection.

Grant: 1R01AI051668-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: BISHAI, WILLIAM R MD
Title: Cell Division Control and Virulence in Mycobacteria
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 2001/09/01-2006/06/30

DESCRIPTION (provided by applicant) Despite more than a century of study the mechanism for slow growth rates in mycobacteria is not understood. A study of mycobacterial regulatory genes led to the isolation of the *Mycobacterium smegmatis* whmD gene which encodes a homologue of the *Streptomyces coelicolor* WhiB protein required for early sporulation control. Unlike its *Streptomyces* homologue, *M. smegmatis* whmD is an essential gene that could only be insertionally inactivated in the presence of a complementing allele in trans. To examine the phenotype of whmD withdrawal, a conditionally expressing whmD mutant was constructed by fusing the complementing whmD gene to the chemically-regulated acetamidase promoter and regulatory gene block. Upon withdrawal of the inducer acetamide, the conditional whmD mutant exhibited irreversible, filamentous, branched growth with diminished septum formation and aberrant septal placement. Nucleic acid synthesis and levels of the essential cell division protein FtsZ were unaltered by WhmD withdrawal. whmD mRNA and WhmD protein were both stably expressed throughout the growth cycle, but became undetectable in late stationary phase. WhmD overexpression resulted in growth retardation and hyperseptation. Together, these phenotypes indicate a role for WhmD in *M. smegmatis* septum formation and cell division. A closely related protein, WhmB, which is a homologue of the *S. coelicolor* WhiD late sporulation regulator, has been shown to be specifically induced in macrophages in vitro and during granuloma formation in vivo in the *Mycobacterium marinum*-frog model. Hence this related gene appears to be a non-essential, in-vivo induced virulence gene in certain mycobacteria. In this proposal we will use complete genomic microarrays and real-time RT-PCR to study the pattern of transcriptional regulation of the whiB-like genes of both *M. tuberculosis* and *M. smegmatis*. Additionally we will characterize alterations in whm gene expression patterns in mycobacterial mutants lacking key regulatory genes (such as sigma factor genes or other members of the whiB- like family). We will evaluate the consequences of genetic deletion of whmB and whmD in *M. tuberculosis* and will use the knockout mutants (or conditionally complemented mutants should the gene[s] be essential) in virulence studies in the mouse model. We also propose a characterization of the biochemistry of *M. smegmatis* WhmD by testing the hypothesis that its 4 cysteines bind a metal ion (such as Zn or Fe), determining which regions of the proteins specify function by genetic domain-swapping experiments and cross-complementation with existing *Streptomyces* whi mutants, and by seeking proteins which interact with WhmD with the yeast 2-hybrid system. We will also pursue the 3-dimensional structure of the *M. smegmatis* and *M. tb*. WhmD proteins and potentially other family members using high resolution NMR. Lastly we will examine the role of WhmD and WhmB in cell physiology by evaluating their sub-cellular localization through GFP tagging and fluorescence microscopy. This latter line of investigation promises to provide important clues as to the roll of WhmD in mycobacterial cell division and the basis for filamentation, branching, and hyposeptation upon genetic withdrawal of whmD expression.

Includes RPGs (Research Project Grants)
Excludes Clinical Trials

Grant: 1R01AI051669-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: ZAHRT, THOMAS C PHD
Title: M.tuberculosis Genes Regulating Persistent Infection
Institution: MEDICAL COLLEGE OF WISCONSIN MILWAUKEE, WI
Project Period: 2001/08/15-2006/05/31

DESCRIPTION (provided by applicant) Tuberculosis is the leading cause of death in the world from a single infectious agent, and is responsible for more than 3 million deaths annually. The high mortality rate in individuals infected with *Mycobacterium tuberculosis* is due in part to its ability to parasitize macrophages and establish long-term, persistent infection in the host despite cell-mediated immunity. Although the current anti-tubercular drug arsenal is effective in treating individuals suffering from active disease, these drugs are ineffective in treating the 2 billion people that currently suffer from latent tuberculosis, or that are infected with multi-drug resistant strains of *M. tuberculosis*. One group of transcriptional regulatory determinants that may play a critical role in processes associated with *M. tuberculosis* latency is the two-component signal transduction systems. These systems mediate adaptation processes and have been shown to contribute to virulence and disease elicitation in other organisms. The goals of this study are to characterize further a two-component system of *M. tuberculosis* (MprA-MprB) that is required for the establishment and maintenance of persistent infection. In this proposal, we plan to: (i) Identify and characterize the genes regulated by the MprA transcription factor. The genes regulated by MprA will be identified from the *M. tuberculosis* chromosome using biochemical enrichment and genetic selection techniques, and will be characterized by gene inactivation, promoter expression analysis, and evaluation in model systems for infection. (ii) Analyze the *in vivo* expression profile of the MprA response regulator, and the genes regulated by MprA. This will be accomplished by expression analysis of these genes using GFP reporter technology, primer extension analysis, molecular beacon technology, and DNA microarray based analysis under physiologically relevant conditions. (iii) Delineate the effects of MprA de-regulation on host-pathogen interactions. This will be accomplished by examining effects of MprA loss or overexpression on *M. tuberculosis* virulence. Virulence studies will include bacterial survival and cytokine expression analysis as assayed in *in vitro* tissue culture systems and animal model systems of infection. These studies will also address the effects of MprA de-regulation on *Mycobacterium bovis* BCG attenuation. The proposal outlined here is expected to improve our understanding of genes required by *M. tuberculosis* for pathogenesis, and help better define the conditions encountered and responses utilized by *M. tuberculosis* during the latent stage of infection. We hope that the analysis of two-component systems will aid in the identification of genetic determinants for which novel anti-tubercular drugs can be developed.

Grant: 1R01AI051929-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: RUBIN, ERIC J PHD
Title: Drug resistance in tuberculosis--Genetic and dynamics
Institution: HARVARD UNIVERSITY (SCH OF PUBLIC BOSTON, MA
HLTH)
Project Period: 2001/09/15-2006/08/31

DESCRIPTION (provided by applicant) Tuberculosis remains a major public health problem worldwide. The management of tuberculosis has recently become increasingly difficult with the rising incidence of drug resistant disease. The causative organism, *Mycobacterium tuberculosis*, is innately resistant to many common antibiotics and accumulates mutations that allow resistance to multiple antibiotics. Resistance now necessitates the use of less potent and more toxic second line drugs for treatment of many infected patients. While the mechanisms of drug resistance to several first line agents has been determined, little is known about second line drugs. We propose to investigate the molecular mechanisms of resistance to both antimycobacterial antibiotics and agents not ordinarily used to treat tuberculosis. We will utilize methods for generating and analyzing transposon mutants that we have recently developed. These methods have already identified previously unknown mutations responsible for drug resistance. We will also study the population dynamics of *M. tuberculosis* during infection in a mouse model. Our mathematical model suggests that drug potency and the physiologic "cost" (effect on growth rate) of resistance are more important in the development of resistance than mutation rate, an observation that would affect the design of antibiotic treatment strategies. We will use "tagged" strains to follow the fate of individual strains, both drug sensitive and drug resistant, during infection. This will allow us to compare the relative fitness of strains and determine the cost of drug resistance in the presence and absence of antibiotic treatment. The results of these studies will be useful for developing new antibiotic treatments and strategies

Grant: 1R01AI051930-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: GRAHAM, JAMES E PHD
Title: Mycobacterium tuberculosis in vivo gene expression
Institution: VANDERBILT UNIVERSITY NASHVILLE, TN
Project Period: 2001/09/17-2006/08/31

DESCRIPTION (provided by applicant) Mycobacterium tuberculosis is a pathogen that is able to adapt to a variety of different environments encountered during the progressive course of human infection. An ability to inhibit maturation of the macrophage phagosome initially created an ideal environment for bacterial growth, allowing colonization of the body. When deprived of this favorable environment by the host's own tissue-damaging immune response, infection often fails to progress, and bacilli enter a nonreplicating latent state, having reached a degree of equilibrium with the host. Changes in the host over time may then allow bacteria to resume replication, leading to further tissue destruction and extracellular growth to high titers. This project will identify bacterial genes that are specifically expressed by *M. tuberculosis* during adaptation to these in vivo environments. A new method developed specifically for examination of mycobacterial mRNAs expressed in infected host cells and tissues (SCOTS) has so far identified 9 *M. tuberculosis* genes which are expressed in response to growth within cultured human macrophage phagosomes. The first aim of the proposed work is to make bacterial strains specifically inhibited in expression of these genes and evaluate their ability to survive and grow in cultured human macrophages. The second aim is to identify additional *M. tuberculosis* genes that are differently expressed by tubercle bacilli in another environment that bacilli normally encounter during the natural course of human infection. Bacterial genomic array hybridization with cDNAs obtained by SCOTS will be used to analyze global mRNA expression patterns in bacilli recovered from patient sputum samples, providing insight into the physiology and metabolism of the microbe during active growth in the human lung. Our third aim is to extend analysis of differential bacterial gene expression to a C57BL/6 mouse model of host interaction, facilitating studies of both active and latent types of infection in a genetically defined host. Bacterial cDNA already obtained by SCOTS from tubercle bacilli growing in cultured mouse macrophages and from infected mouse lung tissues will be compared by array hybridization to cDNA from tubercle bacilli growing in human macrophages and lung tissues. A limited number of *M. tuberculosis* genes commonly expressed in response to these mammalian host interactions will then be evaluated for their contributions to virulence in this animal model. Understanding the roles of such differentially expressed genes will further define host-pathogen interactions in human disease, and allow development of new tools to reduce the enormous global impact of tuberculosis on mankind.

Grant: 1R01AI051975-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: YANG, ZHENHUA MD
Title: Virulence Related Genetic Variation of M Tb Strains
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2001/09/30-2006/07/31

DESCRIPTION (provided by applicant) Tuberculosis (TB) remains the leading infectious cause of global mortality. The emergence of drug resistant TB and a deadly synergy with HIV seen in recent years highlight the need for a better understanding of TB transmission and pathogenesis. The long term objectives of our project are: 1) to identify underlying genetic factors of Mycobacterium tuberculosis important to the pathogenesis and the epidemiology of TB and 2) to provide targets for the development of more efficient vaccine and therapeutical agents for TB prevention and control. Our hypothesis for the present project is that survival of successful bacterial pathogens not only depends on their ability to alter global patterns of gene expression in response to the changing environments during infection, but also depends on the presence or absence of certain genes in the bacteria that play an important role in virulence, and that loss or gain of such genes in clinical strains of M. tuberculosis is associated with their infectivity and pathogenicity. Our specific aims and the related experimental strategies are: 1) to determine genomic variation among M. tuberculosis strains associated with different epidemiological and clinical characteristics by genomic subtraction and to identify DNA unique to epidemiologically and clinically successful strains of M. tuberculosis in comparison with epidemiologically and clinically less successful strains, and with already sequenced strains of M. tuberculosis, H37Rv and CDC 1551; 2) to assess the potential importance of the fragments identified by DNA subtraction to TB transmission and pathogenesis by screening for the presence or absence of the fragments in approximately 700 clinical isolates collected during a five year population-based molecular epidemiological study conducted in Arkansas; and the relative distribution of these subtraction products will be determined by epidemiological and clinical co-variables; and 3) to identify and to characterize genes contained in the subtraction products which are associated with TB transmission and pathogenesis, the flanking regions of the fragments identified by subtraction will be cloned for sequencing. By combining epidemiological information with molecular genetics, we are conducting a focused search for genes associated with transmission and pathogenesis.

Grant: 1R03AI048363-01A1
Program Director: LAMBROS, CHRIS
Principal Investigator: BERMUDEZ, LUIZ E
Title: Target for Mefloquine in Mycobacteria
Institution: CALIFORNIA PACIFIC MED CTR-PACIFIC SAN FRANCISCO, CA
CAMP
Project Period: 2001/04/01-2003/03/31

DESCRIPTION (provided by applicant): Infections caused by mycobacteria are responsible for severe morbidity and mortality. Mycobacterium tuberculosis and Mycobacterium avium are intracellular pathogens that infect both healthy individuals and immunocompromised patients. M. avium are usually resistant to conventional anti-tuberculosis therapy, and with the few drugs shown to have anti-M. avium activity in humans, such as the new macrolides, treatment or prolonged prophylaxis of disseminated disease selects resistant mutants after a course of monotherapy. In addition, multiple outbreaks of multi-drug resistant M. tuberculosis have created clinical challenges for hospital and community management of patients. The goal of this proposal is to be focused and apply new strategies to identify and characterize the targets of mefloquine, a drug just recognized to have activity against mycobacteria. We have found that mefloquine has in vitro activity against both M. avium and M. tuberculosis and is borderline bactericidal against M. avium organisms in mice (we have not tested the activity in vivo against M. tuberculosis). Because mefloquine can achieve tissue concentrations 80-fold greater than serum levels, and mycobacteria survive intracellularly and have a long half-life, this class of compound has potential to become part of anti-mycobacterial regimen. Furthermore, mefloquine is active against M. avium strain resistant to macrolides, quinolones, isoniazid, ethambutol and rifampin, suggesting a novel mechanism of action. Therefore, we believe that determining the biochemical target of mefloquine in mycobacteria can lead the way to developing compounds with even more potent activity. The results thus far obtained with mefloquine suggest that it is the first very active drug identified against mycobacteria in years. Specifically, we plan to: (1) clone the mefloquine resistant-determinant using resistant mutants. In addition, by using a M. avium promoter library cloned in a reporter construct (green fluorescent protein), we plan to determine the pathways in the bacterium that are inhibited or stimulated when M. avium is exposed to mefloquine. This work, focused on an active anti-mycobacterial compound, has the potential to unveil new target(s) in both M. avium and M. tuberculosis.

Grant: 1R03AI049081-01
Program Director: LAUGHON, BARBARA E.
Principal Investigator: SULING, WILLIAM J PHD
Title: DIHYDRONEOPTERIN ALDOLASE, A TUBERCULOSIS DRUG TARGET
Institution: SOUTHERN RESEARCH INSTITUTE BIRMINGHAM, AL
Project Period: 2001/05/01-2003/04/30

DESCRIPTION: The purpose of this pilot research project is to investigate the enzyme dihydroneopterin aldolase (DHNA, EC 4.1.2.25) as a target for therapeutic intervention in disease caused by *Mycobacterium tuberculosis* (MTB). Unlike mammalian cells, which acquire folates exogenously through active transport, MTB and many other bacteria must synthesize folates de novo. DHNA is an enzyme present early in the metabolic pathway for the synthesis of reduced folates from GTP. The absence of DHNA in mammalian cells makes this enzyme an attractive target for chemotherapy. Depletion of reduced folates through inhibition of this pathway leads to inhibition of DNA, RNA and protein synthesis. Comprehensive studies of the folate biosynthetic pathway in MTB are lacking but genes coding for enzymes in this pathway have been identified through the Sanger Centre MTB genome sequencing project. A DNA sequence in the MTB genome data base has been identified tentatively as coding for DHNA. For this pilot study, we propose to establish that the gene listed as *foiX* (emb1 locus MTCY7H7B, accession Z95557.1) and *foiB* (swissprot locus FOLB MYCTU, accession 006275) codes for DHNA. Our objectives will be to clone and express the *foiX/foiB* in *Escherichia coli*, and prove that the protein is functionally DHNA. We will also assess the essentiality of the gene by construction of DHNA-deficient MTB strains. This will be done in MTB by allelic exchange mutagenesis and a counter selection method based upon a mycobacterial thermosensitive origin of replication and toxicity of the *sacB* gene to MTB in the presence of sucrose. The results of this pilot study will enable us to better understand the biochemistry of folate metabolism in MTB. It will also provide purified DHNA for future drug discovery studies based upon structure-activity relationships, molecular modeling and crystallographic structure-based drug design.

Grant: 1R03AI049093-01
Program Director: NEAR, KAREN A.
Principal Investigator: BEHAR, SAMUEL M MD
Title: TREATMENT OF TUBERCULOSIS WITH IMMUNOMODULATORS
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 2001/05/01-2004/04/30

DESCRIPTION: Tuberculosis is the leading cause of death in HIV-infected individuals in the world. Infection with HIV results in defects in cell mediated immunity that increases the severity of tuberculosis following infection, reinfection or reactivation of latent infection by *Mycobacterium tuberculosis*. This application for an R03 grant proposes to develop the immunomodulatory glycolipid a-galactosylceramide as a treatment for tuberculosis in patients with concurrent HIV infection. Our initial experiments have shown that treating mice with a-galactosylceramide, starting one day following intravenous inoculation with *M. tuberculosis*, prolongs their survival compared to administration of the vehicle alone. The main action of a-galactosylceramide is the activation of NKT cells in a CD1d dependent manner. The remarkable conservation of both CD1d and NKT cells in both humans and mice makes the mouse a suitable model to test the efficacy of a-galactosylceramide for the treatment of tuberculosis. The experiments proposed in Aim 1 will confirm and extend our finding that a-galactosylceramide protects mice from tuberculosis after inoculation with *M. tuberculosis* by both the intravenous and aerosol routes. An optimum dosing schedule will be developed and we will assess the efficacy of a-galactosylceramide in the treatment of established tuberculous disease. Additionally, changes in the tissue mycobacterial burden, histopathological features, and immunological parameters in treated and untreated mice will be examined to determine the manner in which a-galactosylceramide modifies the natural history of tuberculosis. In Aim 2, we will determine whether a-galactosylceramide acts synergistically with traditional anti-mycobacterial chemotherapy in the treatment of tuberculosis. We believe that this series of experiments will lay the foundation for the development of a-galactosylceramide as an adjunct therapy for tuberculosis, a treatment that ultimately may be relevant to other infectious diseases as well.

Grant: 1R15AI043929-01A1
Program Director: BAKER, PHILLIP J.
Principal Investigator: CAPORALE, DIANE A PHD
Title: Coinfection of Tick-borne Diseases in Areas of Wisconsin
Institution: UNIVERSITY OF WISCONSIN STEVENS STEVENS POINT, WI
POINT
Project Period: 2001/09/15-2004/09/14

DESCRIPTION (provided by the applicant): Close to 400 human cases of Lyme disease and several --cases of Babesiosis and Human Granulocytic Ehrlichiosis (HGE) are reported in the State of Wisconsin each year. For several years, Lyme disease has been known to be highly endemic in the northwestern region of the state. But little is known about the co-infection rate of these three diseases. Past deer surveys have suggested no existence of deer ticks in the eastern part of Wisconsin, except Marinette County near Green Bay. However, I recently identified deer ticks on mice in the Lower Kettle Moraine, and 3.6 percent were found infected with *Borrelia burgdorferi*. Deer tick populations have progressively been spreading to Central Wisconsin. It is important to investigate more thoroughly the population structure of deer ticks and associated diseases. Study sites in Wisconsin will include Dunn County, Portage County and Marinette County. For three years, adult ticks will be collected in each fall season by dragging flags. The microgeographic distribution of *Ixodes scapularis* will be determined. The 16S rDNA sequence of the ticks will be compared to determine whether gene flow exists among these areas. The DNA from crushed tick parts will also be tested for tick-borne pathogens using the polymerase chain reaction (PCR). The genes targeted will be *OspB* for *B. burgdorferi*, the 16S-like gene for *Babesia microti* and the 16S rRNA gene for *Ehrlichia* (HGE). DNA sequencing for all three pathogens will be used for species verification. The microgeographic distribution and co-infection rates of tick-borne diseases, from their main vector are major contributions to the understanding of the spread of tick-borne diseases in Wisconsin. A Lyme disease vaccine is now available to the public, which is a recombinant form that includes the *ospA* gene of a New York *B. burgdorferi* strain. I recently discovered a highly mutated strain from southeastern Wisconsin. It is possible that the vaccine may not be effective against this strain. I propose to monitor the frequency and spread of this strain in northwestern, central, and northeastern Wisconsin. If this strain is prevalent in this state, vaccine developers should investigate the efficacy of their vaccine on this strain.

Grant: 1R15AI047802-01A1
Program Director: LANG, DENNIS R
Principal Investigator: MELLIES, JAY L PHD
Title: Activation of EPEC Virulence Genes
Institution: REED COLLEGE PORTLAND, OR
Project Period: 2001/07/15-2004/06/30

DESCRIPTION (provided by the applicant): Enteropathogenic *E. coli* (EPEC) is an important cause of diarrhea of infants living in developing countries. EPEC is the prototype organism of a group of pathogenic bacteria that cause intestinal attaching and effacing (AE) lesions. All genes necessary for the AE phenotype are encoded within a 35.6 kb pathogenicity island (PAI) termed the locus of enterocyte effacement, or LEE. A virulence plasmid-encoded regulator, PerABC, was previously described in EPEC. We have shown that the expression of a novel LEE-encoded regulator, Ler, is increased by PerABC. As part of a regulatory cascade, Ler then activates transcription of four polycistronic operons of the LEE, which includes at least 24 of the 41 predicted LEE ORFs. Ler also controls the expression of genes located outside the LEE. Thus, Ler is a novel global regulator of EPEC virulence genes. Our long-term research goal is to understand how appropriate expression of EPEC virulence factors occurs within the human intestine. During this period of support, we will characterize the cis-acting sequences necessary for Ler-mediated activation of the LEE2 and tir promoters, determine whether Ler alone is sufficient for activation of these prototypical promoters, and determine whether Ler affects RNA polymerase binding at LEE2 and tir. Through these genetic and biochemical studies we will obtain a greater understanding of the molecular pathogenesis of EPEC. EPEC and *E. coli* serotype O157:H7, a member of the enterohemorrhagic *E. coli* (EHEC) category of *E. coli*, are related pathogens. In the U.S., EHEC is of particular concern in food safety and public health because this organism has caused many outbreaks of bloody diarrhea due to contaminated meat products, produce and water. Through the study of EPEC virulence gene regulation we will also gain insight into the molecular pathogenesis of EPEC.

Grant: 1R15AI047810-01A1
Program Director: HALL, B. FENTON
Principal Investigator: BERKOWER, CAROL L BA
Title: DEVELOPMENT OF RECOMBINANT BCG VACCINES FOR MALARIA
Institution: TOWSON STATE UNIVERSITY TOWSON, MD
Project Period: 2001/03/15-2006/02/28

DESCRIPTION: (Adapted from Applicant's Abstract) Malaria is a major global health burden with annual incidences of approximately 300 million cases and two to three million deaths per year. Ninety percent of malaria cases occur in tropical Africa, with the majority of deaths among children under 5 years old. Development of a vaccine represents an essential goal for an effective control strategy. However, for a vaccine to have a significant impact it would need to be safe, easy to administer, effective in children, and affordable in Africa. A new method of vaccine development involves the engineering of BCG, the widely used tuberculosis vaccine, into a recombinant vaccine vector (rBCG) capable of expressing foreign antigens. Immunization with rBCG expressing viral, bacterial, or parasitic foreign antigens elicits antibody as well as CD4+ and CD8+ T cells responses to the foreign antigens in mice and monkeys. This project will involve the generation of rBCG strains expressing three pre-erythrocytic antigens from mouse malaria, *Plasmodium yoelii*. Expression of these antigens will be optimized using combinations of promoters and signal sequences. Mice will be vaccinated with the rBCG/malaria strains and challenged with live *P. yoelii* sporozoites. The immunization studies will test the ability of rBCG/malaria vaccines to stimulate an immune response against the *P. yoelii* antigens as well as their ability to protect mice against malaria. Mice will also be immunized with a combination vaccine consisting of several rBCG strains expressing different *P. yoelii* antigens. Finally, based on observations from this work, rBCG strains will be generated expressing the homologous antigens from *P. falciparum*, which causes over 90 percent of deaths from malaria in humans. These strains will be made available for further testing in mice and humans.

Grant: 1R15AI048576-01
Program Director: RUBIN, FRAN A.
Principal Investigator: BUTKO, PETER PHD
Title: NATURAL ANTIBODY AGAINST GROUP B STREPTOCOCCUS IN MICE
Institution: UNIVERSITY OF SOUTHERN MISSISSIPPI HATTIESBURG, MS
Project Period: 2001/04/01-2004/03/31

DESCRIPTION (Verbatim from Applicant's Abstract): Group B streptococcus (GBS, *Streptococcus agalactiae*) is the most common cause of neonatal sepsis and meningitis in the USA. A humoral factor has been discovered in normal mouse serum which protects the animals against GBS infection. It is hypothesized that the protective factor is natural IgM antibody. Two goals of the proposed research are: (i) To confirm that the antibody is of the IgM type and that it is protective against experimental GBS infections in vivo; (ii) to identify the antigenic target for this antibody on the bacterial surface. To these aims, several clinical isolates and mutant strains of GBS, in combination with in vitro enzyme-linked immunosorbent assays (ELISA) and in vivo mouse protection experiments will be utilized. Results obtained with mice as animal model may open a new avenue of investigation into possible role of natural antibodies in human immunity to GBS infection and bacterial infection in general, with possible implications for immunotherapy. The results also may provide impetus to the research on the antigenic targets on GBS, possibly other than the capsular polysaccharide, which would have implications for current GBS vaccine efforts. A non-scientific goal of this project is to increase the intensity of health-related undergraduate research and teaching in a mid-size rural university with no medical school and to increase diversity in the work force in the academic research and medicine.

Grant: 1R15AI049964-01
Program Director: MILLER, MARISSA A.
Principal Investigator: WILKINSON, BRIAN J PHD (BACTERIOLOGY)
Title: Physiology of S aureus Vancomycin Resistance
Institution: ILLINOIS STATE UNIVERSITY NORMAL, IL
Project Period: 2001/08/15-2005/08/14

DESCRIPTION (provided by applicant): It is imperative that serious *Staphylococcus aureus* diseases such as bacteremia and infectious endocarditis are treated aggressively with effective antimicrobial agents. These diseases had mortality rates of 80 and 100 percent respectively in the pre-antibiotic era. Most hospital strains of *S. aureus* are now methicillin-resistant (MRSA), and such strains are typically resistant to multiple other antibiotics. The glycopeptide antibiotic vancomycin was the sole remaining antibiotic to which *S. aureus* remained uniformly susceptible. Recently, vancomycin-resistant strains have arisen in patients during long-term vancomycin therapy (so called glycopeptide-intermediate susceptible *S. aureus* or GISA strains). It is imperative that we understand the mechanism of vancomycin resistance in such strains. Vancomycin-resistant strains have been step selected in my laboratory. It is likely that the resistance mechanism involves several mutations and is multifaceted. I propose to study the mechanism of *S. aureus* vancomycin resistance in laboratory and clinical strains. I will study the composition and structure of peptidoglycan, and teichoic acid and lipoteichoic acid in GISA strains, and attempt to understand the mechanism of decreased autolytic activity observed in such strains. Cell wall alterations appear to be involved in vancomycin resistance. I will attempt to understand the increased NaCl-sensitivity of GISA strains through studying the accumulation of compatible solutes, and whether Na⁺ ions accumulate intracellularly to growth inhibitory levels upon NaCl stress. Limited studies toward understanding the genetic basis of vancomycin will be undertaken. Nucleotide sequence analysis of cloned selected genes that physiological studies indicate may be altered in GISA strains will be carried out. It is expected that these studies will lead to improved methods for the control of methicillin-resistant and vancomycin-resistance *S. aureus* infections, and form the basis for development of novel antistaphylococcal agent.

Grant: 2R21AI021463-11A2
Program Director: LANG, DENNIS R
Principal Investigator: PETERSON, JOHNNY W PHD
MICROBIOLOGY:MICROBIOLOGY-UNSPEC
Title: MOLECULAR MODE OF ACTION OF BACTERIAL ENTEROTOXINS
Institution: UNIVERSITY OF TEXAS MEDICAL BR GALVESTON, TX
GALVESTON
Project Period: 1985/09/01-2002/04/30

DESCRIPTION (Verbatim from Applicant's Abstract): Our overall objective is to define the mechanism of action of cholera toxin (CT). While CT directly upregulates adenylate cyclase activity by catalyzing the ADP-ribosylation of Gs-alpha, thereby increasing 3',5'-adenosine monophosphate (cAMP) levels, equally important are stimulatory effects on arachidonic acid (AA) metabolism leading to increased production of eicosanoids (e.g., prostaglandin E2 [PGE2]). PGE2 stimulates adenylate cyclase, intestinal ion transport, and synthesis of cytokines (e.g., IL-6). Importantly, our recent results showed that CT and the CT B-subunit (CT-B) could stimulate AA metabolism independent of ADP-ribosylation of GS-alpha by signaling the expression of the plaa gene, which encodes phospholipase A2-activating protein (PLAA). After cloning the human plaa cDNA, we hyperexpressed the gene in prokaryotic and eukaryotic systems. Our experiments with plaa antisense oligonucleotides have shown that PLAA is an important participant in the mechanisms by which CT upregulates PLA2. Another novel finding includes the chemical reaction that occurs between PGE2 and imidazole derivatives (e.g., L-histidine). The formation of PGE2-imidazole (-histidine) covalent adducts was confirmed by NMR and mass spectrometry. We demonstrated that these adducts reduced cAMP levels in CT-treated CHO cells and reduced CT-induced fluid loss in a murine intestinal loop model. In this proposal, we will examine the role of PLAA, PGE2, and LTC4 in enhancing the stimulatory effect of cAMP on the secretory response in the small intestine. Intestinal cells (e.g., Paneth cells) forming PLAA in response to CT will be identified using in situ hybridization and immunohistochemistry. Further, the role of PLAA and Paneth cells will be established by using knockout mice. We also propose to define the regulatory mechanisms by which CT signals the rapid synthesis of PLAA mRNA. Using recombinant PLAA and expression of plaa cDNA in eukaryotic cells, we will evaluate its stimulatory effect on PLA2 activity. Increasing knowledge of the molecular events in cholera should lead to future strategies to control the hypersecretion of water and electrolytes.

Grant: 2R21AI026328-12
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: KELLY, KATHLEEN A PHD
Title: T-Cell Mediated Immunity In Chlamydial Genital Infection
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 2001/07/15-2003/07/14

DESCRIPTION (provided by the applicant): Chlamydia trachomatis infection remains a major cause of pelvic inflammatory disease, and often leads to fallopian tube injury and infertility. While effective antibiotics are available, asymptomatic infection may ascend to the upper genital tract (GT) and cause irreversible tissue damage before it is discovered. One strategy for avoiding injury to the upper GT is through preventative vaccination. However, the anti-chlamydial CD4 T helper type 1 (Th1) response that develops to eradicate infection is also thought to damage the upper GT. Preliminary data from our laboratory has found that CD4 cells are primarily recruited to the upper but not the lower GT where infection begins. We have also found that prostaglandin E2 (PGE2) is induced in the upper tract during infection. These data indicate that the upper and lower GT are two immunologically distinct compartments and respond differently during Chlamydia infection. We hypothesize that the recruitment of Th1 CD4 effector cells to the lower GT is insufficient for completely eradicating chlamydiae resulting in upper GT infection. Upper GT infection causes the local production of PGE, a potent inducer of IL-10, which impedes the anti-chlamydial Th1 CD4 response, thereby promoting upper GT injury. To test this hypothesis we will 1) Identify chemokines that control the recruitment of Th1 CD4 cells to the upper and lower GT 2) Determine the effect of altered Th1 CD4 cell recruitment on immunity against Chlamydia infection 3) Identify mechanism(s) whereby IL-10 hampers an anti-chlamydial Th1 CD4 response in the upper GT and 4) Determine if PGE2 influences the anti-chlamydial Th1 CD4 response in the upper GT. To achieve these goals, we will use the mouse model of chlamydial genital infection to manipulate the immune response in BALB/c and various transgenic mouse strains. The results from this proposal will determine if shifting the Th1 CD4 immune response against Chlamydia from the upper to the lower GT can increase protective immunity and reduce tubal pathology. In addition, the role of regulatory factors, such as PGE2/IL-10 will be characterized. By targeting different immune regulatory mechanisms in this process within the upper and lower GT we hope to enhance protective immunity and reduce upper GT injury. If successful, this approach will aid vaccine development and promote strategies that target immune responses to discrete sites within mucosal surfaces.

Grant: 2R21AI034431-06
Program Director: BAKER, PHILLIP J.
Principal Investigator: HAAKE, DAVID A
Title: OUTER MEMBRANE PROTEINS OF PATHOGENIC LEPTOSPIRA SPECIES
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 1996/05/01-2003/04/30

DESCRIPTION (Adapted from the Applicant's Abstract): Leptospirosis is considered to be the most widespread zoonotic disease in the world. New vaccine strategies are needed for prevention of infection by these pathogenic spirochetes belonging to the genus *Leptospira*. The focus of this proposal is to elucidate the structural requirements and mechanisms of leptospiral outer membrane protein (OMP)-mediated immunoprotection. Leptospiral OMPs are important targets of a protective host immune response. When expressed as recombinant membrane proteins, the combination of the leptospiral porin OmpL1, and a surface-exposed lipoprotein, LipL4l are synergistically immunoprotective in the hamster model of leptospirosis. Interestingly, detergent-solubilized His6 fusion forms of these same two proteins are not immunoprotective. The goal of Specific Aim 1 is to determine what aspects of the membrane formulation are essential for the immunoprotective effect. The effects of detergent, adjuvant, protein-structure, and membrane-formulation will be systematically examined. Passive immunization studies will be performed to show that humoral mechanisms are sufficient for protection. Specific Aim 2 will establish in vitro assays of antibody-mediated protection including bactericidal activity, growth inhibition, and opsonophagocytosis. The finding of synergistic immunoprotection will be studied in Specific Aim 3 by testing for OmpL1/LipL4l interactions on the leptospiral surface, and by examining whether OmpLi/LipL4l interactions affect accessibility to antibody binding. In Specific Aim 4, polyclonal and monoclonal antibodies specific for OmpL1 and LipL4l will be tested for relevant biological activity with the goal of identifying the epitopes responsible for OMP-mediated immunoprotection.

Grant: 2R21AI037230-07
Program Director: BAKER, PHILLIP J.
Principal Investigator: MATHER, THOMAS N
Title: ROLE OF TICK SALIVA IN LYME DISEASE AND VACCINE STRATEGY
Institution: UNIVERSITY OF RHODE ISLAND KINGSTON, RI
Project Period: 2001/09/30-2002/09/29

DESCRIPTION (Provided by Applicant): Interactions at the vector-host interface are likely to be the most critical to transmission of many arthropod transmitted infections. Our studies have demonstrated that through the action of their saliva, black-legged ticks (*Ixodes scapularis*) manipulate the host immune response in a manner that both assures blood feeding success, and favors survival and transmission of Lyme disease spirochetes (*Borrelia burgdorferi*). We have learned that these bacteria receive cues from tick saliva to regulate their protein expression, perhaps leading to enhanced invasiveness or survival in the host. We have been successful in discovering several novel molecules, including *I. scapularis*' salivary anti-complement protein (Isac) and a Factor Xa-inhibiting anticoagulant (Ixolaris), and recombinant proteins are in production. Taken together, this progress now allows us to begin testing our hypothesis, that an effective prevention strategy for Lyme disease, and other *I. scapularis*-transmitted infections, can best be developed by manipulating host immune responses to components of vector saliva or saliva-induced microbial products. In continuing this project, we will test whether a protocol integrating vector salivary gland genomics and proteomics can accelerate both discovery and recovery of potentially important immunogenic molecules. Massive cDNA sequencing of an *I. scapularis* salivary gland cDNA library containing full-length clones has already revealed nearly 1,200 sequences and at least 476 genes. We will begin expressing these sequences in recombinant baculovirus expression systems or cloning them into bacterial plasmids to generate candidate protein and DNA vaccines. In addition, recent technological advances in *B. burgdorferi* genomics are allowing rapid progress to be made on studies examining *B. burgdorferi* gene and protein expression in the presence and absence of tick saliva, or under other starvation-stress conditions. We will test whether whole genome analysis by DNA arrays and 2-D gel electrophoresis can facilitate discovery of potential immunogens. Candidate vaccines will be screened for their ability to interrupt tick feeding or block pathogen transmission in a white-footed mouse (*Peromyscus leucopus*) model. We expect that these studies will lead us to develop vaccination strategies that combine tick and bacterial elements for preventing Lyme disease, and possibly a broader range of tick-transmitted infections.

Grant: 2R21AI041682-04A1
Program Director: RUBIN, FRAN A.
Principal Investigator: ENGLEBERG, N CARY MD
Title: Mucoïd Variants of S Pyogenes Exhibit Enhanced Virulence
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 1997/09/01-2003/03/31

DESCRIPTION (Verbatim from Applicant's Abstract): Invasive disease caused by Group A streptococci (GAS) continues to be an infrequent but often devastating medical problem in humans. Antibiotics and surgery are the only mainstays of treatment, because our understanding of the pathogenesis of these infections is insufficient to design rational interventions and vaccines. Our research group is studying a signal transduction system in GAS that controls the expression of several genes required for development of invasive skin infection in mice. The streptococcal proteins CsrS and CsrR comprise a two-component regulatory system. CsrS is a membrane-spanning sensor-kinase molecule that presumably phosphorylates CsrR, a DNA-binding regulator. Phosphorylated CsrR binds upstream of specific genes and represses expression of hyaluronic acid capsule, streptolysin S, pyrogenic exotoxin B, streptokinase, and mitogenic factor. Mutation of *csrR* results in bacteria with enhanced expression of these virulence factors and dramatically more severe skin lesions in mice. Our laboratory will use genetic methods to determine how the CsrRS system functions and whether spontaneous mutations in this locus affect the outcome of human disease. 1) We will analyze the contributions of key CsrR-regulated factors in the development of dermonecrotic skin lesions in order to define which factors are necessary for aggressive disease. 2) We will determine how the phosphorylation of CsrR correlates with the expression of virulence factors at various points in the bacterial growth cycle. Because preliminary data show that *csrS* mutants persistently repress CsrR-regulated virulence genes, we will study both the kinase and phosphatase activity of CsrS independently to determine how its activity relates to the phosphorylation of CsrR. 3) *csrS* mutants produce larger, more mucoid colonies when grown in a 5 percent CO₂ atmosphere instead of air, and the bacteria are more virulent than wild type. We propose to determine the basis for the phenotypic change and to determine whether it accounts for the enhanced virulence. 4) We have observed that spontaneous mutations in *csrRS* occur often in vivo. When this occurs, there is synergy between the mutant and wild type strains at the site of infection that enhances bacterial growth, local invasion, and bacteremia in mice. We will use strains with two copies of *csrRS* to evaluate the basis for this synergy in mice in order to avoid the spontaneous emergence of mutants during wild type infections. 5) Finally, we propose to study human isolates from patients with invasive and non-invasive streptococcal infections to determine whether subpopulations of *csrRS* mutant bacteria are a common feature of these life-threatening diseases. If so, this observation will have major impact on the future analyses of human cases.

Grant: 1R21AI043970-01A2
Program Director: MILLER, MARISSA A.
Principal Investigator: WILKINSON, BRIAN J PHD (BACTERIOLOGY)
Title: STAPHYLOCOCCAL VANCOMYCIN AND METHICILLIN RESISTANCE
Institution: ILLINOIS STATE UNIVERSITY NORMAL, IL
Project Period: 2001/09/30-2003/09/29

DESCRIPTION (Adapted from the Applicant's Abstract): It is imperative that serious Staphylococcus aureus diseases such as bacteremia and infectious endocarditis are treated aggressively with effective antimicrobial agents. These diseases had mortality rates of 80 and 100 percent respectively in the pre-antibiotic era. Most hospital strains of S. aureus are now methicillin-resistant (MRSA), and such strains are typically resistant to multiple other antibiotics. The glycopeptide antibiotic vancomycin was the sole remaining antibiotic to which S. aureus remained uniformly susceptible. Recently, vancomycin-resistant strains have arisen in patients during long-term vancomycin therapy (so called glycopeptide-intermediate susceptible S. aureus or GISA strains). It is imperative that we understand the mechanism of vancomycin resistance in such strains. Vancomycin-resistant strains have been step-selected in the investigators laboratory. It is likely that the resistance mechanism involves several mutations and is multifaceted. The investigators propose to study the mechanisms of S. aureus vancomycin and methicillin resistance in laboratory and clinical strains, and their work is organized into three major lines of investigation: I. Phenotypic studies of vancomycin resistance; II. Genotypic studies of vancomycin resistance; and III. Stress gene and protein expression in methicillin and vancomycin resistance. In I, they will study the composition and structure of peptidoglycan, teichoic acid and lipoteichoic acid in GISA strains, and attempt to understand the mechanism of decreased autolytic activity observed in such strains. They will attempt to understand the increased NaCl-sensitivity of GISA strains through studying the accumulation of compatible solutes, and whether Na⁺ ions accumulate intracellularly to growth inhibitory levels upon NaCl stress. In II, they will attempt to discover the genetic basis of vancomycin resistance through transposon mutagenesis studies, direct cloning of genes responsible for vancomycin resistance, and cloning and nucleotide sequence analysis of target genes as indicated to play a role in vancomycin resistance through studies in I above. In III, they will study the role of cell wall antibiotic stress gene and protein expression in methicillin and vancomycin resistance through a proteomics approach, transcription profiling using gene chip technology, and by selective capture of transcribed sequences. It is expected that these studies will lead to improved methods for the control of methicillin-resistant and vancomycin-resistance S. aureus infections, and form the basis for development of novel antistaphylococcal agents.

Grant: 1R21AI045541-01A2
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: CHAKRABARTY, ANANDA M BA
Title: NUCLEOTIDE SYNTHESIS AND METABOLISM IN MYCOBACTERIA
Institution: UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL
Project Period: 2001/09/30-2003/03/31

DESCRIPTION (Adapted from the Applicant's Abstract): Many of the mycobacterial cell wall polymers are derived from basic building blocks of nucleoside diphosphate-sugar residues such as GDP-mannose and UDP-galactose which require nucleoside triphosphates (NTPs) such as GTP or UTP for each molecule of the sugar residue present in the polymer. Nothing is known about how the precursor NTP pool in the mycobacterial cells is regulated. The Investigator has recently isolated 4 different proteins from *Mycobacterium smegmatis* that modulate the specificity of nucleoside diphosphate kinase (Ndk) to primarily synthesize either GTP, or UTP, or CTP. Whether such proteins may play a role in supplying specific NTPs for cell wall/envelope components is not known at present. The investigators propose to characterize Ndk-complexing proteins in *M. bovis*-BCG, isolate their genes, and attempt to make knockout mutations to examine if loss of the gene functions may result in altered cell wall synthesis as characterized chemically. They have also recently reported the secretability of two ATP-utilizing enzymes (Ndk and ATPase) of *M. bovis* BCG. The secretion of these two enzymes is facilitated in the presence of eukaryotic proteins such as bovine serum albumin or ovalbumin. Preliminary evidence suggests that the presence of receptors on the surface of *M. bovis* BCG cells that bind ovalbumin. They propose to isolate and characterize these receptors. Additionally, they would like to make deletion and site-directed mutations in the *ndk* gene of *M. bovis* BCG to examine the role of putative sequences (motifs) in the secretability of Ndk.

Grant: 1R21AI047142-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: OWEN, WHYTE G PHD BIOCHEMISTRY
Title: KATG PEROXIDASE IN ISONIAZID ACTIVATION AND RESISTANCE
Institution: MAYO CLINIC COLL OF MEDICINE, ROCHESTER, MN
ROCHESTER
Project Period: 2001/09/30-2003/09/29

DESCRIPTION (Adapted from the Applicant's Abstract): Tuberculosis is one of the leading causes of death worldwide from an infectious disease. Isoniazid is a front line antibiotic used in the treatment of tuberculosis. The Mycobacterium tuberculosis heme catalase/peroxidase KatG is responsible for activating isoniazid to a reactive biocidal species. Antibiotic resistance to isoniazid is a growing concern and can occur by deletions or point mutations in the katG gene. One of these, a serine to threonine substitution at position 315, KatG(S315T) is found in greater than 50 percent of clinic isolates resistant to isoniazid. The investigators' research seeks to understand the mechanism of isoniazid activation and molecular basis for drug resistance caused by the S315T and other point mutations in KatG using biochemical and spectroscopic techniques. KatG will be purified from recombinant and native sources and the activities and spectroscopic properties compared. Besides a catalase/peroxidase activity, KatG can also catalyze several other reactions including Mn²⁺ peroxidase, P450-like oxygenase, and peroxynitritase activities. Which of these or possibly other redox reactions are responsible for isoniazid oxidation and activation will be tested using wild-type and mutant KatG proteins to investigate the mechanism of activation. Optical, EPR, NMR, and resonance Raman spectroscopies are revealing subtle differences in the heme active site of wild-type KatG and KatG 315T). These spectroscopic techniques will be applied to various forms of wild-type and mutant enzymes in order to elucidate the molecular basis for reactivity toward isoniazid and the reduced rate of isoniazid oxidation by KatG(S315T). NMR relaxation measurements and x-ray crystallography are being used to map the isoniazid binding site on both enzymes to determine whether subtle differences in distance and/or orientation are responsible for the reduced turnover of drug by the mutant enzyme. Steady-state and rapid kinetic techniques will follow ligand-binding rates to the heme iron and the formation and decay of reactive intermediates in the catalytic cycle for both wild-type KatG and KatG(S315T) to determine whether the S315T mutation affects one of the steps in the catalytic cycle. EPR spectroscopy and spin-traps are being used to trap reactive intermediates. The stable products formed in this reaction are being characterized by mass spectrometry to reveal chemical information about the nature of intermediates in the reaction of isoniazid oxidation by KatG. Site directed mutagenesis of residues so implicated in isoniazid resistance other than S315 will be generated and the effects on enzyme activities and spectroscopic properties examined to determine structural information about the mutant enzymes. Analogs of isoniazid will be used as additional biochemical and spectroscopic probes of the reaction mechanism and molecular basis for drug resistance.

Grant: 1R21AI047744-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: SHERMAN, DAVID R PHD
Title: Hypoxia, Latency and Reactivation in M tuberculosis
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2001/09/30-2002/12/31

DESCRIPTION (provided by the applicant): Central to the pathogenic success of Mycobacterium tuberculosis (MTB) is its ability to persist within humans for long periods in a latent state, without causing any overt disease symptoms. Roughly one-third of the world population harbors latent MTB, greatly complicating efforts at tuberculosis control. A person with latent tuberculosis has about a 10 percent lifetime chance of developing active disease, and when such a person contracts HIV, the risk of developing reactivation TB increases to 8 - 10 percent per year. Hypoxic conditions within the human host are widely regarded as crucial for development of latent tuberculosis, but the MTB adaptive response to hypoxia is at present very poorly understood. The goal of this proposal is to define the MTB hypoxic response as it relates to latency and reactivation. The α -crystallin protein of MTB is powerfully induced by hypoxia and has been implicated in long-term survival. Alpha-crystallin will be used as a model system to determine the mechanisms by which oxygen tension control MTB gene expression. In addition, specific conditions in which Alpha-crystallin is necessary for achieving latency and reactivation will be determined. Finally, with Alpha-crystallin as an example, other proteins important for the adaptation to and from hypoxia will be identified and characterized. The result will be better tools to confront the threat to more than one billion persons with latent tuberculosis, millions of whom are now or will soon be co-infected with the human immunodeficiency virus, HIV.

Grant: 1R21AI047880-01A1
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: TOTTEN, PATRICIA A
Title: Hemolysin and Immunobiology of Chancroid
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2001/04/01-2003/03/31

DESCRIPTION (Verbatim from Applicant's Abstract): *H. ducreyi* is the causative agent of chancroid, a disease characterized by genital ulcers, and in 50 percent of the cases, inguinal lymphadenopathy. The occurrence of chancroid outbreaks in the United States coupled with its association with the heterosexual transmission of HIV in Africa makes understanding the pathogenesis of this disease imperative so that rational intervention strategies can be devised. We have developed a primate model for chancroid that measures the effect of disease progression from the pustular to the ulceral to the resolution stages of disease at a genital site in an animal closely related to humans. We now intend to use the primate model to study the local and systemic immune response induced by infection with *H. ducreyi* and the immunobiology of chancroidal disease. We hypothesize that a predominant Th1 response will be induced and will be correlated with clearance of the organism from genital tissues. We have previously shown that *H. ducreyi* produces a toxin, which has been termed a hemolysin, based on its ability to lyse red blood cells, although its role in pathogenesis undoubtedly depends upon its ability to affect other cells important in chancroidal lesions. We have shown that immunization with hemolysin increases the clearance of a homologous strain of *H. ducreyi* from lesions in the temperature-dependent rabbit model and now intend to study the nature of immune response that enhances clearance of this organism from genital tissues in the primate model. Thus, we propose to study the ability of immunization with hemolysin (compared to immunization with *H. ducreyi* cell envelopes) to attenuate lesion development and enhance clearance of *H. ducreyi* from genital ulcers. We also propose to study the effect of immunization on the systemic and local immune response, localization of *H. ducreyi* in primate lesions, cellular and antibody response to individual antigens, and the possible mechanism of protection by antibodies from immunized primates. We have previously shown that the target cell range of hemolysin includes keratinocytes, fibroblasts, lymphocytes, and macrophages and hypothesize that hemolysin enhances ulcer development, evasion of the immune response in chancroidal disease, and survival of *H. ducreyi* in genital lesions. Thus we will study the contribution of hemolysin to lesion progression and survival of *H. ducreyi* in primate genital ulcers and the effect of hemolysin expression on the local and systemic immune response. These studies will provide a better understanding of the role of the *H. ducreyi* hemolysin in the pathogenesis and immunobiology of chancroid and will provide a groundwork on which to base future strategies for vaccine development for chancroid.

Grant: 1R21AI048437-01
Program Director: LAUGHON, BARBARA E.
Principal Investigator: HEIFETS, LEONID B MD
Title: NEW CLASS OF ANTITUBERCULOSIS AMIDES:PRECLINICAL DEVELOP
Institution: NATIONAL JEWISH MEDICAL & RES CTR DENVER, CO
Project Period: 2001/08/15-2002/07/31

The resurgence of tuberculosis (TB) coupled with the emergence of multi-drug resistant strains (MDR-TB) necessitates that novel therapies be developed. One strategy for the discovery of novel drugs is to identify the targets of effective agents. Pyrazinamide (PZA), one such agent, has a unique sterilizing activity. It is the inclusion of PZA with isoniazid and rifampin in current treatment regimens that constitutes the basis for 6-month short course therapy for *M. tuberculosis* (Mtb). PZA efficacy depends upon conversion of PZA to pyrazinoic acid (POA), the active agent. The lack of pyrazinamidase, an enzyme that converts PZA to POA, confers resistance to PZA. PZA is effective only against Mtb among the mycobacterial species, and then is effective only at an acidic pH. A recent study demonstrated that acidic pH enhances the intracellular accumulation of POA and suggested that *M. tuberculosis*, unlike other mycobactenar species, lacks an efficient POA efflux mechanism. Despite the clinical importance of pyrazinamide, there has been no integrated, comprehensive investigation of this compound. Although the mechanism of PZA activation and resistance is known, the precise mycobacterial function that is inhibited by POA remains unknown. Using 5-Cl-PZA as a selective agent for mycobacteria, we have found that 5-Cl-PZA and PZA inhibit fatty acid synthetase I FASI in Mtb. Preliminary studies have shown improved activity of PZA analogs against Mtb in infected human monocyte-derived macrophages and in murine models of infection. This integrative study proposes further elucidation of the mechanism of FASI inhibition to guide the generation of new analogs of PZA. We will test the activity of improved new compounds against PZA-resistant Mtb and against other species of mycobacteria and correlate this with FASI inhibition. We will compare the activity of new agents to PZA against intracellular bacilli (in macrophages), and assess the toxicity in human peripheral blood monocytes and cultured hepatocytes. Introduction of improved PZA analogs should result in more rapid sterilization of Mtb in a murine model of tuberculosis and therefore potentially lead to a shorter course therapy when tested in humans.

Grant: 1R21AI048590-01
Program Director: LANG, DENNIS R
Principal Investigator: PICKETT, CAROL L PHD
Title: CAMPYLOBACTER JEJUNI CYTOLETHAL DISTENDING TOXIN
Institution: UNIVERSITY OF KENTUCKY LEXINGTON, KY
Project Period: 2001/09/30-2002/09/29

DESCRIPTION (Adapted from the Applicant's Abstract): *Campylobacter jejuni* is currently the most common bacterial cause of diarrheal disease in humans in the United States. It has also recently been strongly implicated as one of the most common infections to precede the development of Guillain-Barre syndrome. Thus far, only one *C. jejuni* toxin, cytolethal distending toxin (CDT), has been definitively proven to exist. Recent work has shown that the *C. jejuni* CDT is a member of a new family of toxins that can cause a G2 cell cycle block in certain mammalian cultured cells. However, little is known about the specific functions of this toxin and how it contributes to *C. jejuni* disease. The long-term goals of this proposal are to understand how CDT causes a cell cycle block and to relate this understanding to disease. In particular, the investigator plans to examine the functional roles of 3 CDT proteins (CdtA, CdtB, and CdtC) in causing the G2 block. The proposal seeks to elucidate the target(s) of CDT in sensitive cells, and determine the roles of the Cdt proteins in causing the toxic effect. The specific aims of the proposal are to test the hypothesis that one or more of the Cdt proteins function to bring about the G2 block in various types of eucaryotic cells. In Aim 1, a variety of specific mutations will be introduced into each of the Cdt proteins. The effects of the mutations on toxicity and on selected other properties will be tested. In Aim 2, yeast 2-hybrid techniques will be used to identify HeLa cell proteins that interact with Cdt proteins. In Aim 3, a yeast expression system will be exploited to identify how Cdt causes the G2 block. In Aim 4, a HeLa cell expression system will be used to complement and extend the studies in the first 3 aims. In summary, these studies are designed to increase our understanding of CDT and the mechanism by which it causes a G2 block.

Grant: 1R21AI048622-01
Program Director: LANG, DENNIS R
Principal Investigator: LIBBY, STEPHEN J PHD
Title: THE SLYA REGULON IN SALMONELLA PATHOGENESIS
Institution: NORTH CAROLINA STATE UNIVERSITY RALEIGH, NC
RALEIGH
Project Period: 2001/09/27-2003/09/26

DESCRIPTION (Adapted from the Applicant's Abstract): SlyA is a global transcriptional regulator of Salmonella genes. SlyA is essential for Salmonella pathogenesis, for growth within phagocytes, and for resistance to oxidative stress. SlyA is maximally expressed in stationary phase and in macrophages. The focus of this proposal is to discern the mechanism by which SlyA contributes to Salmonella pathogenesis and oxidative stress resistance. The three specific aims are: 1) to identify Sly-regulated genes, 2) to determine the role of SlyA-dependent genes in oxidative stress resistance, and 3) to perform a molecular analysis of slyA transcription and translation.

Grant: 1R21AI048743-01
Program Director: RUBIN, FRAN A.
Principal Investigator: ASHBAUGH, CAMERON D MD
Title: GROUP A STREPTOCOCCAL HYALURONATE LYASE
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 2001/09/30-2003/09/29

DESCRIPTION (Verbatim from Applicant's Abstract): In the past two decades, there has been a resurgence of serious group A streptococcal (GAS) infections throughout the world. The clinical presentation of these infections has included both aggressive primary disease and the post-infectious syndrome of rheumatic fever. Acute invasive infections are characterized by invasion of the organism from superficial to deep foci, the frequent development of hemodynamic instability (streptococcal toxic-shock), and significant morbidity and mortality, often in previously healthy individuals. No single bacterial determinant appears to be uniquely associated with GAS virulence. Indeed, it is likely that the pathogenesis of GAS infection depends on the carefully regulated expression of a number of virulence factors. Because hyaluronic acid is an important component of human extracellular matrix, and because bacteria must negotiate the extracellular space during invasive infection, one long-standing candidate for a bacterial factor contributing to the pathogenesis of invasive GAS disease is hyaluronate lyase, an enzyme that depolymerizes hyaluronate. Although it was recognized many years ago that GAS can express hyaluronate lyase, the gene encoding the enzyme, the nature of its expression in GAS, and the demonstration of its role in virulence has not been established. Preliminary work in this laboratory has identified the chromosomal gene encoding the GAS hyaluronate lyase. The goals of this proposal are to characterize expression of the hyl gene in GAS strains representing prevalent serotypes recovered from invasive GAS disease and to determine the role of the hyl gene product in GAS virulence using several animal models that in sum capture the diverse clinical manifestations of serious human infection. The identification and characterization of novel GAS virulence determinants is a critical component in the continuing effort to understand and prevent GAS pathogenesis.

Grant: 1R21AI049157-01A1
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: CHAN, JOHN R MD
Title: TNF-alpha in Tuberculosis
Institution: YESHIVA UNIVERSITY BRONX, NY
Project Period: 2001/08/01-2002/07/31

The mechanisms by which latent tuberculosis is established and subsequently reactivates are poorly understood. We have used the low dose model of persistent murine tuberculosis to examine the effects of tumor necrosis factor (TNF)-alpha on host immune response in the quiescent phase of infection. Results of these studies demonstrate that persistently infected mice undergo reactivation when neutralized for TNF-alpha by the administration of the monoclonal antibody MP6- XT22. This reactivation is characterized by increased mortality associated with severe inflammatory reaction in the lungs despite only a moderate tissue bacterial load. The severe pathology may result from progressive disorganization of the granulomas and unfocused infiltrating cells in the lungs. In addition, expression of interleukin (IL)-10 is enhanced in TNF-alpha neutralized, persistently infected mice. TNF-alpha and IL-10 can modulate the expression of chemokines and chemokines receptors, molecules that play a critical role in cell migration. Fibronectin-bound TNF-alpha can anchor trafficking T cells thereby halting migration. IL-10 has been reported to block inflammatory molecule-triggered chemokine receptor switch and uncouple signaling through these receptors in monocytes. Consequently, IL-10 can render inflammatory monocytes unresponsive to specific chemokines. Therefore, we propose that TNF-alpha neutralization in persistently infected mice results in aberrant cell migration secondary to dysregulation of localized chemokine and chemokine receptor expression. In addition, the TNF-alpha neutralization may lead to deficiency in the formation of TNF-alpha- fibronectin complex, thereby altering cell trafficking. Finally, enhanced IL-10 expression in TNF-alpha neutralized mice may cause aberrant cell migration because of its ability to modulate the expression and uncoupling of chemokine receptors. The goal of this proposal is to rigorously test these hypotheses. Results obtained from these studies should enhance our understanding of the effects of TNF-alpha on the host immune response in persistent infection, particularly those pertaining to cell migration to and within the granuloma and pathology. This, in turn, will illuminate the mechanisms by which TNF-alpha exert its antimycobacterial effects in persistent infection.

Grant: 1R21AI049424-01
Program Director: AULTMAN, KATHRYN S.
Principal Investigator: MUNDERLOH, ULRIKE G PHD ENTOMOLOGY AND
PARASITOLOGY
Title: Development of Paratransgenic ticks for Disease Control
Institution: UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN
Project Period: 2001/09/30-2002/09/29

DESCRIPTION (provided by applicant): Tick-borne diseases are increasingly being diagnosed in humans and animals. Some are due to the resurgence of previously known illnesses, like Rocky Mountain spotted fever, but others are due to new, emerging pathogens. Among the spotted fever-group (SFG) alone, 8 new human pathogens have been described in the last 15 years, but they also include viruses, ehrlichias and Babesia. Novel, efficient, specific and environmentally acceptable methods that interfere with disease transmission by ticks are urgently needed. Using paratransgenic ticks that carry specific symbiotic prokaryotes expressing an antimicrobial substance, as has been achieved with the symbiote of the Chagas disease vector, *Rhodnius prolixus*, could offer a safe and effective way to reduce disease transmission by ticks. A major obstacle to accomplishing this goal has been the lack of culture systems for tick symbiotes. We have in our laboratory the largest collection of tick cell lines: We have successfully used these to isolate tick symbiotic rickettsiae from the Lone Star tick (Isolate MOAa) and the Rocky Mountain wood tick (*R. peacockii* isolate -DAE100R}. We have characterized these microbes by light and electron microscopy by using specific -antibodies, as well as by PCR and nucleotide sequence analysis of 16S rDNA and other key genes. We are now in the process of defining the cultures to facilitate genetic manipulation of the symbiotes. Our long term aim is the stable transformation of *Rickettsia Peacockii* with the cecropinA an insect porin gene, infection of ticks with the transformed rickettsia, and interference with pathogen transmission. We plan to target the non-functional *rompA* gene of *R. peacockii* as a site for homologous transformation, avoiding deleterious effects associated with disruption of a vital gene, e.g., the *rpoB* gene. We will take the recent advances in the successful transformation of insect-borne rickettsiae as a guide specifically we will 1): optimize culture conditions for production of *R. peacockii* in tick cell culture, verify identity of the isolate as *R. peacockii* by molecular analysis, and examine its behavior in mammalian and tick cell culture by light and electron microscopy.-2) We will characterize and analyze *R. peacockii* in vivo in ticks in terms of tissue tropisms and transstadial/transovarial passage. Finally, we will work towards 3. stable transformation of *A. peacockii* with cecropina will then test the transformants for antimicrobial activity in vitro and in ticks, and by sequence analysis.

Grant: 1R21AI049571-01
Program Director: QUACKENBUSH, ROBERT L.
Principal Investigator: KAVATHAS, PAULA B
Title: Characterization of Human T Cells Against Chlamydia
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 2001/09/30-2003/09/30

DESCRIPTION (provided by the applicant): Chlamydia trachomatis (Ct) is the most common cause of bacterial sexually transmitted disease worldwide. In previous work, we identified epitopes in the major outer membrane (MOMP) that are recognized by CD4+ T cells in the context of HLA class II molecules and others that are recognized by CD8+ T cells in the context of HLA class I molecules. Most recently, we used HLA A2 tetramers to identify MOMP-specific CD8+ T cells in blood of Ct-infected individuals and found that they killed Ct infected epithelial cells. We will extend these studies to other individuals using HLA class I tetramers made with other allotypes. To identify and characterize MOMP-specific CD4+ T cells isolated from blood, we will create HLA class II tetramers using MOMP epitopes that are recognized by CD4+ T cells. We will characterize both CD8 and CD4 MOMP-specific T cells with regard to cell surface proteins that are involved in homing (e.g. integrins and chemokine receptors), function (e.g. cytokines, perforin) and state of differentiation (e.g. HLA-DR, CD62L) by using multi-color flow cytometry with monoclonal antibodies against cell surface and intracellular proteins. Comparisons of lymphocytes from the blood vs. sites of infection will provide information on how representative blood-derived samples are of cells at infected sites. Lastly, the ability of MOMP-specific CTL to limit the increase of infectious Ct in infected genital tract cells and the effects of mutations in CTL epitopes on such limitation will be determined. The ability to detect and characterize human anti-Chlamydial T cells with tetramers and cellular reagents of defined HLA- and epitope specificity would be useful for vaccine development, especially since most of the epitopes are in conserved regions of MOMP.

Grant: 1R21AI050216-01
Program Director: SIZEMORE, CHRISTINE F.
Principal Investigator: REMOLD, HEINZ PHD
Title: A Novel Mechanism of Innate Immunity Against TB
Institution: BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA
Project Period: 2001/09/01-2002/08/31

DESCRIPTION (provided by the applicant): Tuberculosis (TB) persists as a global health concern due to high prevalence of infection and drug resistance. More detailed knowledge of TB pathogenesis is needed to unravel novel approaches for prevention and treatment. Early antimicrobial mechanisms which are part of the innate immune response system are crucial for the outcome of the infection with *Mycobacterium tuberculosis* (Mtb). In this application, we investigate a novel mechanism, how human macrophages (Mp), the primary host cell of Mtb, inhibit growth of Mtb when they undergo apoptosis. Our preliminary data show that apoptosis of the Mp infected with Mtb is associated with their capacity to exhibit strong anti-mycobacterial activity, whereas necrosis promotes extracellular bacterial growth. We further showed that virulent Mtb are able to avoid host Mp apoptosis, whereas the attenuated Mtb strain H37Ra strongly induces apoptosis. We postulate that Mp-apoptosis 1) restricts Mtb growth by sequestering the bacilli within apoptotic bodies and 2) packages Mtb for rapid and efficient elimination by freshly recruited phagocytes. Uptake of free Mtb is also associated with arrested phagosome maturation and unrestricted intracellular growth. We think that Mtb packaged in apoptotic bodies are eliminated more effectively by the defense systems of the Mp. We will examine possible cooperative effector systems when uninfected Mp are presented with Mtb contained in apoptotic bodies. We have also found that Mtb-induced Mp apoptosis and associated anti-mycobacterial activity are dependent on the concerted action of tumor necrosis factor α , cytosolic phospholipase A2, and on intra-cellular Calveins, but the specific role and function of these mechanisms is not understood. We will investigate the role of these mechanisms in induction of apoptosis and anti-mycobacterial activity and how attenuated and virulent Mtb differ in the activation of these processes. The goals, thus, are to 1) determine how avirulent Mtb induce apoptosis and antimycobacterial mechanisms and how virulent Mtb avoid it, 2) to find out how apoptotic Mp block growth of Mtb and 3) to define the anti-mycobacterial mechanisms of naive Mp after uptake of apoptotic infected Mp.

Grant: 2R37AI010085-37
Program Director: RUBIN, FRAN A.
Principal Investigator: DALE, JAMES B MD
Title: Chemistry and Immunology of Streptococcal M Proteins
Institution: UNIVERSITY OF TENNESSEE HEALTH SCI MEMPHIS, TN
CTR
Project Period: 1996/06/01-2006/05/31

DESCRIPTION (provided by the applicant): The overall goal of this project is to develop a safe and broadly effective vaccine that will prevent group A streptococcal infections. Previous studies have shown that the surface M proteins, which are the major protective antigens, contain tissue-crossreactive epitopes as well as protective epitopes. The serotype-specific protective epitopes may be separated from potentially harmful autoimmune epitopes by using limited N-terminal peptides of M proteins. The protective M protein fragments representing multiple serotypes of group A streptococci may then be combined to form a multivalent vaccine. The specific aims of this proposal are: 1) To identify the primary structures of M proteins or other surface proteins that contain opsonic (protective) epitopes from serotypes of group A streptococci that are epidemiologically important and, therefore, necessary vaccine components, 2) To construct recombinant, multivalent vaccines that evoke optimal opsonic antibody responses in laboratory animals against 26 different serotypes of group A streptococci, 3) To test immune rabbit sera evoked by multivalent vaccines for opsonic and bactericidal antibodies against clinical isolates of group A streptococci collected from children with pharyngitis in 10 geographic sites in the U.S., 4) To develop strategies of intranasal delivery of multivalent M protein-based vaccines that result in secretory and systemic immune responses, and 5) To directly compare the protective immunogenicity of multivalent M protein-based vaccines delivered to mice via either the intramuscular or intranasal routes. In our preliminary studies, we have identified six epidemiologically important serotypes of group A streptococci that are not opsonized by antisera against the N-terminal M protein peptides. We propose a series of experiments to determine the covalent structures of the M proteins, M-like proteins, or other surface proteins that contain opsonic epitopes so that these M serotypes may be included in multivalent vaccines. We will construct a 26-valent vaccine composed of 4 different recombinant, hybrid proteins. The individual hybrid proteins will be tested for protective and tissue-crossreactive immunogenicity after intramuscular injection of rabbits. Because mucosal delivery of streptococcal vaccines may have both immunological and practical advantages over parenteral delivery, we will assess different strategies of intranasal delivery and then directly compare the protective efficacy of i.n. vs i.m. vaccines in mice. The studies should provide the detailed information needed to develop a safe and effective multivalent vaccine that could prevent the majority of streptococcal infections in North America and Western Europe.

Grant: 2R37AI024424-16
Program Director: BAKER, PHILLIP J.
Principal Investigator: BARBOUR, ALAN G MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC
Title: MOLECULAR BASIS OF BORRELIA PATHOGENESIS
Institution: UNIVERSITY OF CALIFORNIA IRVINE IRVINE, CA
Project Period: 1986/12/01-2006/03/31

DESCRIPTION (provided by the applicant): Relapsing fever *Borrelia* infections provide an excellent experimental system for comprehensively understanding the pathogenesis strategy of antigenic variation and the systems biology and evolution of an infectious disease. The long term goals of the project are to (a) define the molecular basis for antigenic variation and pathogenesis by relapsing fever *Borrelia* spp., (b) characterize host responses that select for antigenic variants and other evasion strategies by the parasite, (c) delineate the organismal and population biology of relapsing fever spirochetes in their mammalian reservoirs and arthropod vectors, (d) investigate the evolution of antigenic variation of relapsing fever *Borrelia* spp., and (e) integrate the findings from studies for goals (a) to (d) through mathematical modeling and computer simulation. Specific aim 1: Genetic basis for antigenic variation. The first hypothesis for this aim is that antigen switches are the result of a form of homologous recombination that repairs a break at the expression site with an archived gene. To test this hypothesis recombination-deficient mutants of *B. hermsii* will be produced. The second hypothesis is that the organization of the archival genes and non-coding repetitive sequences in part determines switch rates. We will investigate this by further characterizing the sequences of archival sites. The third hypothesis for this aim is that the diversity of the variable membrane protein genes is largely attributable to intragenic recombination within a single strain and not through the accumulation of point mutations or lateral gene transfer. We will test this by molecular evolution analyses of the expressed and archival variable genes in different strains of *B. hermsii* and in different species of *Borrelia*. Specific aim 2: Structure-function studies of variable membrane proteins. For this aim further crystallographic studies of the Vsp proteins and their mutants will be carried out and work on the structure of one or more Vip proteins will begin. The hypothesis is that while the major structural features of the Vsp and Vip proteins are maintained across the respective repertoires, significant differences in antibody reactivities and host cell interactions by the proteins are determined by local structural polymorphisms. The general approach to this hypothesis is to further characterize functional differences between different Vsp and Vip proteins under in vitro and in vivo conditions and to examine the effect of mutations on functions and antibody interactions of the proteins. Specific aim 3: Biology of parasitism of mammalian hosts by relapsing fever spirochetes. The major hypothesis for this aim is that the parameters and dynamics of experimental relapsing fever will eventually be sufficiently elucidated at the molecular to organismal levels that an infection can be accurately simulated by computers. We will evaluate this by developing models that are based on currently available data about infection dynamics and then use these early models' deficiencies to direct us in further experimentation and model building.

Includes RPGs (Research Project Grants)
Excludes Clinical Trials

Includes RPGs (Research Project Grants)
Excludes Clinical Trials

Grant: 2R37AI032956-10
Program Director: MILLER, MARISSA A.
Principal Investigator: PALZKILL, TIMOTHY G PHD
Title: Beta Lactamase Mutations in Antibiotic Resistance
Institution: BAYLOR COLLEGE OF MEDICINE HOUSTON, TX
Project Period: 1992/07/01-2006/04/30

DESCRIPTION (provided by the applicant): Bacterial resistance to antimicrobial agents has increased in recent years and now represents a significant threat to successful antibiotic therapy. One example of this phenomenon is the development of resistance to B-lactam antibiotics. B-lactam antibiotics, such as the penicillins and cephalosporins, are among the most frequently used antimicrobial agents. The most common mechanism of resistance to B-lactam antibiotics is the production of B-lactamases, which cleave the antibiotic, rendering it harmless to bacteria. Based on primary sequence homology, B-lactamases have been grouped into four classes. Classes A, C and D are active-site serine enzymes that catalyze, via a serine-bound acyl-enzyme intermediate, the hydrolysis of the B-lactam antibiotic. Class B enzymes require zinc for activity and catalysis does not proceed via a covalent intermediate. Because of the diverse range of substrate specificities of these enzymes, virtually all B-lactam antibiotics are susceptible to hydrolysis. Clearly, the design of new antibiotics that escape hydrolysis by the growing collection of B-lactamase activities will be a challenge. It will be necessary to understand the catalytic mechanism and basis for substrate specificity of each class of B-lactamase. The goal of this work is understand how the amino acid sequence determines the structure, catalysis, and substrate specificity of the IMP-I B-lactamase of class B and the P99 class C B-lactamase. This will be achieved by randomizing amino acid positions in the active-site pocket of each enzyme to sample all possible amino acid substitutions. All of the random substitutions will then be screened to identify those substitutions that alter the substrate specificity of the enzyme. Enzymes containing substitutions that alter substrate specificity will be purified and characterized biochemically. The sets of random substitutions will also be screened using phage display methodology to identify residues critical for catalysis. A further goal of this proposal is to use the detailed knowledge of the interface between B-lactamase inhibitory protein (BLIP) and B-lactamase, in combination with random mutagenesis and phage display, to create derivatives of BLIP that bind and inhibit B-lactamases and penicillin binding proteins. The new BLIP derivatives will be characterized biochemically and structurally. The information gained from these studies will be useful for the rational design of new antibiotics and inhibitors.

Grant: 2R37AI033713-09
Program Director: TAYLOR, CHRISTOPHER E.
Principal Investigator: IGLEWSKI, BARBARA H PHD
MICROBIOLOGY:BACTERIOLOGY
Title: REGULATION OF PSEUDOMONAS AERUGINOSA PROTEASES
Institution: UNIVERSITY OF ROCHESTER ROCHESTER, NY
Project Period: 1993/01/01-2005/12/31

DESCRIPTION (adapted from the investigator's abstract): This proposal is a competing renewal of AI 33713. The long-term goal of this research is to determine the role of quorum sensing in the pathogenesis of *Pseudomonas aeruginosa*. We have shown that *P. aeruginosa* makes three signal molecules (autoinducers); PAI-1, PAI-2, and PQS. PAI-1 and PAI-2 activate the R-proteins LasR and RhlR respectively, to stimulate expression of numerous virulence factor and secondary metabolite genes. These two R proteins belong to the growing family of autoinducer responsive transcriptional regulators found in many Gram-negative bacteria. Homology searches of the genome show that *P. aeruginosa* contains two other R protein genes. Despite the advances made in understanding these important regulatory systems many questions remain. The current proposal seeks answers to some of these questions and has the following specific aims: 1. Continue studies on PAI-1 and PAI-2 by producing and characterizing polyclonal and monoclonal antibodies to these autoinducers. 2. Continue studies on the structure and function of LasR and RhlR. 3. Determine if the other two R proteins in *P. aeruginosa* PAO are involved in quorum sensing and the regulation of known virulence factors including proteases. 4. Continue studies on the role of LasR-PAI-1 and RhlR-PAI-2 in virulence of *P. aeruginosa*, using a model of acute pneumonia in adult mice and collaboratively in a mouse thermal injury model. We will examine the effect on bacterial virulence of mutations in QS genes and determine if antibodies to PAI-1 or PAI-2 alter the outcome of these *P. aeruginosa* infections. The results of these studies will further our understanding of virulence regulation in *P. aeruginosa* and provide new insights into quorum sensing systems in general. They will determine the feasibility of novel approaches to therapy for Gram-negative infections and provide useful reagents for future studies.

Grant: 1U01AI049921-01
Program Director: LANG, DENNIS R
Principal Investigator: PAULSEN, IAN T PHD
Title: Comparative Genomic Analysis of Clostridium perfringens
Institution: INSTITUTE FOR GENOMIC RESEARCH ROCKVILLE, MD
Project Period: 2001/07/02-2004/12/31

DESCRIPTION (Applicant's Abstract): Clostridium perfringens has a long history of being associated with a high level of morbidity and mortality in battlefield and clinical settings, due to its ability to produce a wide range of deadly toxins and cause diseases such as gas gangrene and enteric infections. C. perfringens has also been tested as a potential biological warfare (BW) agent in World War II and the Iraqi government is suspected of attempting to develop C. perfringens as a BW agent before the Gulf War. Because most of the genes encoding the toxins have been cloned and sequenced, it is feared that these may be used to produce these toxins on a large scale and inflict casualties on military and civilian populations. In addition, C. perfringens is genetically tractable and is capable of producing a heat resistant spore; these qualities make it an inviting target for developing BW methods for producing and delivering deadly toxins. The information obtained from genomic sequencing of C. perfringens will permit the development of vaccines and diagnostic reagents that can be used to combat and diagnose the disease caused by this bacterium. The genetic sequence will also allow production of DNA microarrays, to determine which genes are turned on in infections and identify host specificity factors that differentiate human and veterinary pathogenic strains. The genome sequence will also provide important information on fundamental biological processes such as spore production and basic metabolism in the Clostridia.

Grant: 1U01AI050909-01
Program Director: RUBIN, FRAN A.
Principal Investigator: TETTELIN, HERVE S PHD
Title: Genome Analysis of Streptococcus agalactiae
Institution: INSTITUTE FOR GENOMIC RESEARCH ROCKVILLE, MD
Project Period: 2001/09/30-2003/09/29

DESCRIPTION (provided by applicant): Group B streptococci (GBS) or *Streptococcus agalactiae* are weakly beta-hemolytic, facultatively anaerobic Gram-positive cocci, which have emerged over the past 50 years as the most significant bacterial cause of neonatal sepsis, pneumonia, and meningitis. GBS account for 30-50 percent of neonatal bacterial infections and increases in adult GBS infections have also been noted. This demonstrates that GBS infections remain an important public health problem. We propose to sequence the 2.1 Mb genome of *S. agalactiae* serotype Ia strain A909 using a genome-wide random shotgun approach. We will then use the final assembled sequence and its complete annotation to perform detailed comparative genomics analyses between serotype Ia and other organisms causing pneumonia and meningitis. These analyses will allow to identify virulence determinants shared by all organisms or specific to individual ones. These determinants will be related to Drs. Jones and Rubens' experiments on signature tagged mutagenesis and TnpH₂ translational fusion (identification of secreted proteins) mutant libraries constructed through transposon insertions in strain A909. Regions where transposons inserted will be aligned to the genome sequence to identify the genes affected. A subset of the mutants conserved across serotypes will be analyzed in the rat sepsis model to evaluate their virulence. This collaborative effort will provide extensive preliminary data for future proposals relevant to understanding the pathogenesis of *S. agalactiae* infections.

Grant: 1U01AI050942-01
Program Director: BAKER, PHILLIP J.
Principal Investigator: MADAN, ANUP PHD
Title: Sequence of Rickettsia Rickettsii Genome
Institution: INSTITUTE FOR SYSTEMS BIOLOGY SEATTLE, WA
Project Period: 2001/09/30-2002/09/29

DESCRIPTION (provided by applicant): The specific aims of this proposal are to sequence and annotate the genome of *Rickettsia rickettsii*, and to begin functional analysis of the genome. *R. rickettsii* is an obligately intracellular Gram negative bacterium that is widely distributed in the Americas. It is the etiologic agent of Rocky Mountain spotted fever, the most severe of the human rickettsioses. This microorganism has been considered as a potential biological weapon because of its high infectivity, since it causes disease with a short incubation period and relatively high mortality, especially in untreated cases. Currently, a serious discrepancy exists between the potential threat to human health posed by *R. rickettsii* and the number of laboratories studying this important pathogen. To add to existing methodologies presently available for study of this bacterium, this proposal will introduce genomic sequencing as a means to gain valuable insight into the intricacies of the pathogenesis of this microorganism. The derived database will lay the groundwork for further functional proteomic studies of *R. rickettsii* that may facilitate identification of new vaccine candidates and potential therapeutic targets. Additionally, this new information will refine our understanding of the biology of the evolution of this bacterium and perhaps result in recognition of novel pathogenic mechanisms used by *R. rickettsii*. We propose to complete the sequencing and primary annotation of the *R. rickettsii* genome within one year at a total cost of \$0.36 per base pair. An initial computational approach will be used to identify putative open reading frames (ORF) encoding the proteins and genes expressed as RNAs. A newly developed Website will be constructed and maintained to display the *R. rickettsii* genome sequence and its annotation. Metabolic pathways and their components in *R. rickettsii* will be identified by computational analysis of operon structures and comparisons with proteins and pathways in *R. prowazekii* and other bacteria. To facilitate experimental functional genomic analyses of *R. rickettsii*, individual ORFs will be PCR amplified, cloned, expressed, catalogued, and made available to the scientific community. Biochemical functions of selected genes of *R. rickettsii* will be experimentally confirmed. Finally, selected transcriptional regulons of *R. rickettsii* which respond to external signals will be characterized.

Grant: 2R01AR020358-26

Program Director: SERRATE-SZTEIN, SUSANA

Principal Investigator: STEERE, ALLEN C MD INTERNAL
MED:CONNECTIVE TISSUE
DISEASES

Title: LYME ARTHRITIS- A NEW EPIDEMIC DISEASE

Institution: NEW ENGLAND MEDICAL CENTER BOSTON, MA
HOSPITALS

Project Period: 1987/07/01-2006/02/28

DESCRIPTION: (Verbatim) - About 10 percent of patients with Lyme arthritis have persistent knee swelling for months or even several years after oral and intravenous antibiotic therapy. After treatment, such patients, in our experience, have no remaining spirochetal DNA in synovial tissue or joint fluid, suggesting that live spirochetes have been eliminated from the joint. During the past grant cycle, we identified a possible autoimmune mechanism that may partially explain the persistence of Lyme arthritis after antibiotic therapy. The mechanism in DRB1*0401-positive individuals involves molecular mimicry between the dominant T cell epitope of *B. burgdorferi* outer-surface protein A (OspA165-173) and a homologous sequence of human lymphocyte function associated antigen-1 (hLFA-1alpha332-340). In this proposal, we test the hypothesis that synovial inflammation may persist in treatment-resistant arthritis patients with a range of MHC alleles because of molecular mimicry between this dominant T cell epitope of OspA and hLFA-1. Our plan is to determine the frequencies of various MHC alleles in patients with treatment-resistant arthritis compared with those in treatment-responsive patients and those in a control population. PBL and synovial fluid lymphocytes (SFL) from treatment-responsive and treatment-resistant patients will be screened for reactivity with OspA165-173 and LFA-1alpha332-340, and cloned OspA165-173-reactive T cells from selected patients with a range of MHC alleles will be tested for reactivity with hLFA-1alpha332-340. Using an in vitro peptide binding assay, DR or DQ molecules obtained from selected patients' EBV-transformed B cells will be tested for their ability to bind the OspA and hLFA-1 peptides. Finally, after appropriate antibiotic treatment, the efficacy and safety of DMARD therapy will be observed in treatment-resistant patients. Lyme arthritis is the only human form of chronic inflammatory arthritis in which the triggering agent, immunogenetic susceptibility, and a candidate autoantigen are known. Thus, it is currently the only human system in which it is possible to explore specific infectious and autoimmune mechanisms that may lead to chronic inflammatory arthritis.

Grant: 2R01AR040445-12

Program Director: SERRATE-SZTEIN, SUSANA

Principal Investigator: BENACH, JORGE L PHD
MICROBIOLOGY:BACTERIOLOGY

Title: Borrelia burgdorferi. Invasion and Chronicity

Institution: STATE UNIVERSITY NEW YORK STONY BROOK STONY BROOK, NY

Project Period: 1990/05/01-2006/04/30

DESCRIPTION (provided by applicant): In the past funding period, we have shown that there are critical associations between spirochetes and fibrinolytic proteases of the plasminogen activation system. We have shown how these organisms use plasmin to promote their own invasiveness. The *Borrelia* binds plasminogen on their surface where it is activated to plasmin by the plasminogen activator urokinase (uPA). In turn, uPA can be bound to its cell receptor, the urokinase plasminogen activator receptor (uPAR, CD87). *Borrelia burgdorferi* can also induce the upregulation of uPAR on the membrane and the release of the soluble isoform into the medium from peripheral blood monocytes, and from monocyte-like human cell lines. Patients with Lyme disease also have elevated levels of soluble uPAR in the serum and in the cerebrospinal fluid. The function of the soluble uPAR in body fluids in disease, particularly infection, is not known. The hypothesis of this competitive renewal application is that soluble uPAR has an effect on the levels of free uPA, leading to a procoagulant state, which is, in turn, a means to control bacterial dissemination. In this manner, soluble uPAR is a molecule with anti infection properties. The Specific Aims of this proposal are: to determine the biochemical nature of the soluble uPAR released from monocytes after exposure to *Borrelia* in experimental situations; and, to determine the cellular source and biochemical nature of uPAR in human and experimental infection, and to characterize the clinical conditions of uPAR production. The hypothesis will be tested by several biochemical and molecular approaches, and by animal and clinical studies. This proposal embodies extensive experimental evidence that other macromolecules of the PAS, namely, the uPAR (CD87), could also play a critical role in modulating spirochetal infections. There are also two murine models of spirochetal infection can be used effectively in transgenic animals. Furthermore, the availability of transgenic mice for most of the known components of the fibrinolytic system is advancing our knowledge of this system in areas that had not been previously envisioned. For this proposal, we have in vivo correlates (both at the experimental and clinical levels) to the in vitro observations. Specifically, our fully correlated in vitro and in vivo systems will permit translational conclusions within this funding period.

Grant: 2R01AR043520-07
Program Director: SERRATE-SZTEIN, SUSANA
Principal Investigator: BUDD, RALPH C
Title: Gamma Delta T Cells in Lyme Arthritis
Institution: UNIVERSITY OF VERMONT & ST AGRIC COLLEGE BURLINGTON, VT
Project Period: 1994/09/30-2006/05/31

DESCRIPTION (provided by applicant): Lyme disease is the most common vector-borne disease in the United States. It is caused by the spirochete *Borrelia burgdorferi* and transmitted by Ixodes ticks. This renewal application examines the contribution of synovial gamma delta T lymphocytes in Lyme arthritis. Preliminary studies show that gamma delta T cells of the V delta 1 subset accumulate in Lyme arthritis synovial fluid (10-15 percent) and proliferate vigorously in response to lipidated but not delipidated *Borrelia* proteins in the presence of dendritic cells (DC) and IL-2. This may occur through Toll-like receptor 2 (TLR2), which is known to bind *Borrelia* lipoproteins. The synovial V delta 1 clones recognize the MHC class I-like molecules MICA and CD1b and express high and prolonged levels of surface Fas-ligand (FasL). Finally, we have observed that DC contain very high levels of the Fas death receptor inhibitor, FLIP, and are very resistant to Fas-induced death. In fact, Fas ligation on DC actually promotes upregulation of B7.1, B7.2, and CD40, similar to TNF alpha. We have identified a signal pathway for this in which high levels of FLIP can divert signals to the MAP kinase, ERK, and NF-KB by binding to adaptor proteins that link to these pathways. In this manner death signals can be switched to signals for cell growth or proliferation. The model emerging from the preliminary studies is that lipoproteins from *B. burgdorferi* bind to TLR2 on DC to upregulate molecules such as MICA and CD1b that are stimulatory for synovial V delta 1 cells. The V delta 1 cells express high and prolonged surface FasL which is lytic to some synovial components, but may be stimulatory toward DC due to their high expression of FLIP. Each of the three aims studies one aspect of this model. Aim 1 examines whether *B. burgdorferi* activates V delta 1 cells directly or indirectly through TLR2. We will examine cells expressing or not expressing TLR2 for their ability to activate V delta 1 clones in the presence of *Borrelia* proteins, either lipidated or delipidated. Aim 2 studies two important aspects of synovial V delta 1 clones: how their high expression of surface FasL is regulated, and whether the actual gamma delta TCR is responsible for the response to MICA and CD1b. Aim 3 will study development of DC from CD34+ precursors using a newly developed in vitro culture technique using Flt3 ligand. This will be paralleled by studies of FLIP expression, the ability of soluble FasL to promote cell death or growth/differentiation, depending on the levels of FLIP. The FasL stimulated DC will be analyzed for surface induction of CD1b, CD40, B7.1/2, MICA, and their ability to stimulate the V delta 1 clones.

Grant: 2R01AR044415-05
Program Director: TYREE, BERNADETTE
Principal Investigator: HOOK, MAGNUS A
Title: Collagen Binding MSCRAMMs
Institution: TEXAS A&M UNIVERSITY HEALTH SCIENCE COLLEGE STATION, TX
CTR
Project Period: 1996/12/01-2006/04/30

Attachment of bacteria to the host tissue represents the first critical step in a process that may lead to clinically manifested infections. Extracellular pathogens attach via specific surface located MSCRAMMs (Microbial Surface Components Recognizing Adhesive Matrix Molecules) to components of the extracellular matrix. We have previously demonstrated that many Gram-positive bacteria can adhere to collagen and we identified collagen binding MSCRAMMs on *S. aureus* and *E. faecalis* called CNA and ACE, respectively. We found that these MSCRAMMs can bind to multiple sites in collagen. The structure of the collagen binding domain of CNA was determined using X-ray crystallographic methods and found to contain a collagen binding extended trench on its surface. Furthermore, we found that vaccination of mice with recombinant CNA protected the animals against *S. aureus* induced septic death. These results form the basis for the now proposed studies where we want to identify additional collagen binding MSCRAMMs and determine the structures of CNA and ACE. We hypothesize that these MSCRAMMs and other collagen adhesion receptors such as the integrins, contain trenches on their surface that represent the collagen binding sites. This hypothesis, as well as its mechanistic implication for collagen binding, will now be examined. We will identify binding sites in collagens for different adhesion receptors and characterize the interaction of synthetic triple helix collagen peptides containing these binding sites with the receptors. Finally, we will analyze in detail the established role of CNA as a virulence factor in septic arthritis and locate the protective epitopes in this MSCRAMM.

Grant: 2R01AR045386-04
Program Director: SERRATE-SZTEIN, SUSANA
Principal Investigator: HUBER, BRIGITTE T PHD GENETICS:GENETICS
OTHER
Title: Chronic Lyme Arthritis: Is this Autoimmunity?
Institution: TUFTS UNIVERSITY BOSTON BOSTON, MA
Project Period: 1998/09/20-2006/05/31

DESCRIPTION (provided by applicant): Lyme disease is a multifaceted illness, initiated upon infection with the spirochete *Borrelia burgdorferi* (Bb). One manifestation of the disease is arthritis that can become a debilitating, chronic disease in genetically susceptible individuals. During the tenure of this grant, a candidate auto-antigen, hLFA-1, was identified which may elicit an autoimmune response in T cells by molecular mimicry with outer surface protein A (OspA) of Bb. These data form the basis for the current proposal. I. The analysis of the inflammatory T cell response will be expanded and refined by: a) single cell sorting of OspA-reactive T cells from Lyme arthritis patients, using cytokine secretion/capture assays to identify and clone OspA-reactive T cells, regardless of epitope fine specificity. The T cell response of patients in the acute and the chronic phase of the disease will be compared that will provide insights into the mechanism of treatment resistant Lyme arthritis. b) Analyses of OspA responses in HLA.DRB1 transgenic mice. DRB1*0401 and *0101 are associated with susceptibility to chronic Lyme arthritis, while the presence of *1101 seems to protect from this disease. To study the underlying mechanism, the OspA immune response in Bb infected C3H mice expressing these human DRB chains will be mapped and compared. c) testing hLFA-1/DRB1*0401 transgenic mice as an animal model for chronic Lyme arthritis. These mice will be infected with Bb and screened for the development of a chronic arthritic disease. II. The contribution of Abs to the inflammatory joint disease will be assessed. A prominent OspA-specific Ab response is observed during the chronic phase of the disease, correlating with the onset of prolonged bouts of arthritis. These Abs will be tested for an autoimmune reaction, and their involvement in joint inflammation will be determined by: a) the identification of immune complexes in synovial lesions of Lyme arthritis patients; b) the use of OspA Abs from chronic Lyme arthritis patients as probe for autoantigen; c) Bb infection in FcRn-deficient, hLFA-1/ DR*0401 transgenic mice: FcRn-/- mice clear serum IgG fast and are protected from Ab-mediated autoimmune diseases. III. A new candidate for molecular mimicry of OspA, hMAWD-BP, will a) be tested for cross reaction with OspA specific T cells and Abs, b) its aa sequences in the OspA-homologous regions of human and mouse will be compared; c) if significant differences are observed, hMAWD-BP transgenic mice will be prepared; finally, d) Genbank searches will be carried out for the identification of other human homologues of bacterial OspA. These experiments are designed to elucidate the mechanism of treatment resistant Lyme arthritis.

Grant: 1R01AR047058-01A1
Program Director: SERRATE-SZTEIN, SUSANA
Principal Investigator: BOCKENSTEDT, LINDA K MD
Title: CD1-Mediated Immunity Of Lyme Disease
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 2001/04/05-2006/03/31

DESCRIPTION (provided by the applicant): Lyme disease is a multisystem disorder caused by infection with the tick-borne spirochete, *Borrelia burgdorferi*. The disease occurs in stages that reflect the biology of the spirochete as it adapts from survival in the tick to the more hostile environment of the mammal. *B. burgdorferi* contains an abundant array of highly immunogenic outer membrane lipoproteins (Osps) that are differentially expressed throughout the spirochete lifecycle. Lipoproteins incite inflammation and are believed to be the principal spirochete component causing disease. Lipoproteins can also elicit protective humoral immunity but current vaccines fail to elicit long-lived immunity. This proposal is based on our novel findings that absence of CD1d, a non-classical antigen-presenting molecule that binds lipid Ag, renders mice susceptible to *B. burgdorferi* infection and disease. Pathology correlates with high-titer T-dependent antibody responses to spirochete lipoproteins, including lipoproteins only transiently expressed by spirochetes establishing infection in the host. We hypothesize that CD1d functions to facilitate the elimination of spirochetes and their disease-inciting lipoproteins by potentiating innate immune mechanisms. This proposal seeks to 1) understand the molecular and cellular mechanisms by which CD1d-mediated immunity controls spirochetal pathogens; 2) determine whether blocking CD1d will enhance the duration of lipoprotein vaccine-induced immunity; 3) examine the evolution of *B. burgdorferi* infection in the absence of CD1d, and effects the early strong adaptive immune response has on spirochetes that persist in the host; and 4) use the adaptive immune response that evolves in the absence of CD1d to identify key lipoproteins necessary for tick-borne spirochetes to infect and disseminate in the mammalian host. The completion of these studies will allow for broad insights into the role of CD1d in host defense against pathogens and clearance of their proinflammatory lipoprotein antigens that incite disease. In addition, these studies may suggest feasible strategies to improve vaccination-induced protective immunity. For the field of Lyme disease, defining the key molecules expressed by tick-borne spirochetes will move us one step closer toward understanding how this pathogen invades, disseminates and persists in the mammalian host.

Grant: 1R01AR048331-01
Program Director: TYREE, BERNADETTE
Principal Investigator: WHITTUM-HUDSON, JUDITH A
Title: Pathogenic mechanisms in chlamydial reactive arthritis
Institution: WAYNE STATE UNIVERSITY DETROIT, MI
Project Period: 2001/09/28-2006/08/31

DESCRIPTION (provided by applicant): Reactive arthritis (ReA) is a chronic disease characterized by periods of flare alternating with periods of less active inflammation. One criterion of disease classification is a history of prior sexually transmitted or GI infection. Recent data from our collaborative group have shown that a significant number of ReA patients have metabolically active chlamydia in their synovial tissues. To facilitate study of ReA, we developed a murine model of genital chlamydial infection using a human strain of *Chlamydia trachomatis* to study the inflammatory/immunopathologic mechanisms of joint inflammation. Mice develop ascending genital infection within three weeks. Synovial tissue from knees of these animals exhibits histologic signs of synovitis including increased synovial lining cells, dilated vessels, and foci of mononuclear inflammatory cells, and is PCR positive for chlamydial genes. This model will extend our previous data from a murine ocular chlamydial infection model that demonstrated both dissemination of chlamydia to synovial tissue and development of synovitis. The model will allow us to test mechanisms in chlamydia-associated spondyloarthropathy (ReA) relating to the initiation of synovial inflammation and to determine the roles of local and distant host responses in development of persistent chlamydial infection. Specific molecular, microbiologic and immunologic questions to be asked with the murine model are guided by questions raised by the group's clinical findings from ReA patients: (1) what are the roles of pro-inflammatory and immunologic cytokines produced in synovial tissue during the inflammatory processes? (2) what host cells are infected and where does chlamydial persistence begin? (3) what is the metabolic state of chlamydia in those cells? (4) are persistently infected host cells (presumed mononuclear cells/macrophages) the stimulus for inflammation, or do responses by uninfected host cells to the latter provide the inflammatory stimulus? (5) do the locally produced cytokines determine the establishment of inapparent/persistent chlamydial infection in synovial tissue? The proposed studies in a noninvasive murine model of reactive arthritis will extend our clinical and laboratory findings from patients with chlamydia-associated reactive arthritis, and under controlled experimental conditions, enable us to determine the host cells vs chlamydial components which predispose to development and chronicity of joint inflammation. In the long term, these studies will lead to improved therapies for patients with reactive arthritis and/or indicate requirements to prevent this spondyloarthropathy.

Grant: 1R03AR047936-01
Program Director: GRETZ, ELIZABETH
Principal Investigator: KANANGAT, SIVADASAN PHD
MICROBIO/IMMUNOLOGY
Title: IL-1 and IL-1ra Stimulation of S aureus Replication
Institution: UNIVERSITY OF TENNESSEE HEALTH SCI MEMPHIS, TN
CTR
Project Period: 2001/09/28-2004/08/31

DESCRIPTION (provided by applicant): Septic arthritis /bacterial arthritis is a serious complication of rheumatoid arthritis (RA) and other forms of arthritis. It can also occur in seemingly normal joints. If it is not rapidly diagnosed and treated, it can lead to acute joint destruction, osteomyelitis, sepsis and even death. Staphylococcus aureus is the predominant organism invading the RA joints. Synovium of active RA joints contains many cytokines amongst which is the members of the IL-1 family namely, IL-1 alpha, IL-1beta and IL-1 receptor antagonist(IL-1ra). We have observed and previously reported that S. aureus is able to use IL-1beta and IL-1ra as growth factors. Furthermore, these molecules specifically bind to the receptors on the surface of S. aureus (referred hereafter as IL-1R). A major and minor epitope on IL-1beta has been identified to reside in the amino acid residues 208-240 and 118-147 respectively. We hypothesize that specific epitopes in IL-1beta and IL-1 ra bind to IL-1 receptor as a first step in initiating receptor post-receptor mediated events which result in stimulation of replication of the bacterium. Four specific aims will address this hypothesis as follows: 1) Isolate and characterize the S. aureus IL-1R; 2) Clone the S. aureus IL-1 R ; 3) Determine the structural requirements of IL-1beta for binding to S. aureus IL-1R and 4) Determine the structural requirements of IL-1ra necessary for binding to S. aureus IL-1R. This study identifies and characterizes a previously unknown mechanism by which S. aureus utilize members of the IL-1 family as growth factors to defeat the host defense response against infection and persistent inflammation. This particular study is very relevant to septic arthritis in RA and non-RA patients in whom the rapid diagnosis and appropriate treatment to protect the joint structures are very important. A better understanding of how bacteria such as S. aureus use IL-1 family of cytokines to replicate and defeat the host defense is highly relevant to the study of septic arthritis.

Grant: 1R21AR048056-01
Program Director: GRETZ, ELIZABETH
Principal Investigator: MAYES, MAUREEN D MD
Title: Study of Persistent Infection in SSc Skin and Vessels
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX
HOUSTON
Project Period: 2001/09/26-2002/01/14

DESCRIPTION (provided by applicant): The overall objective of this proposal is to study the possibility that in some systemic sclerosis patients, a persistent bacterial infection involving dermal microvascular endothelium or other cells that are resident in skin results in the obliterative microvasculopathy and/or the fibrosing features of this disease. As a first step in addressing this issue, we will test the following hypothesis: persistent bacterial infection of skin or microvasculature occurs more commonly in systemic sclerosis cases than in matched controls and participates in the disease process. Specific aims are: (1) to test skin biopsies from 60 systemic scleroderma patients and 30 matched normal controls for evidence of bacterial persistence by pan-bacterial and chlamydia-specific molecular screening; (2) to microdissect dermal vessels from these same cases and controls and test this tissue by panbacterial and chlamydia-specific molecular probes; (3) to prepare PBMC'S from these individuals and screen with these probes; and (4) depending on positive results, to perform immunohistochemistry studies for these organisms on skin biopsies/vessels from selected patients and appropriate controls. Scleroderma small vessel vasculopathy shares some key features with large vessel atherosclerosis, a condition also characterized by intimal proliferation and luminal narrowing among multiple other abnormalities. Inflammation may play an important role in the pathogenesis of atherosclerosis raising the possibility of infectious agents as mediators in this process. There are several examples of infection resulting in chronic inflammatory autoimmune diseases including Lyme disease (*Borrelia burgdorferii*), and reactive arthritis (ReA), an inflammatory joint disease associated with prior infection by a number of specific bacterial pathogens, including *Chlamydia trachomatis* and various species of the Genera *Salmonella*, *Yersinia*, *Campylobacter*, and others. This research team is comprised of individuals with expertise in clinical scleroderma, the vascular abnormalities of primary and secondary Raynaud's disease, and autoimmunity related to persistent bacterial infections with relevant pathogens. If positive results are obtained in at least a subset of scleroderma cases, intervention trials could be devised with therapy targeted to specific organisms.

Grant:	1R21AR048513-01	
Program Director:	GRETZ, ELIZABETH	
Principal Investigator:	MONTGOMERY, RUTH R	BA
Title:	Host-Spirochete Interaction by Amplification	
Institution:	YALE UNIVERSITY	NEW HAVEN, CO
Project Period:	2001/09/28-2003/07/31	

DESCRIPTION (provided by applicant): We propose to study aspects of host response to the Lyme spirochete, *Borrelia burgdorferi*, using the recently described technique, isothermal rolling circle amplification (RCA). RCA is a new and extremely sensitive method of signal amplification that extends our level of detection to a single antigen- antibody complex or to a point mutation in a nucleotide sequence. We will take advantage of this to examine both protein antigens and nucleic acid signals in biological fluids and in tissues. In Aim 1, we propose to detect multiple strains of *Borrelia burgdorferi* in patient synovial fluids using RCA to amplify the minute signal from each spirochete. In Aim 2, we will build on the results of initial experiments, to use in situ immunoRCA to examine the functional state of macrophages in *B. burgdorferi*-infected mice. We have examined the interaction of macrophages with *B. burgdorferi* in some detail and have shown that in vitro, the cells easily ingest and kill spirochetes, yet in vivo, spirochetes are not cleared from the host, leading to later complications of Lyme disease. The functional state of tissue macrophages in murine skin, the main reservoir for spirochetes in the mammalian host, will be determined by in situ isothermal RCA through analysis of their cytokine profiles over a time course before, during, and after dissemination of *B. burgdorferi*. In summary, this proposal employs a novel and powerful technique to address several possible mechanisms of spirochetal evasion of the immune system.

Grant: 1R21AT000412-01
Program Director: WEST, NEAL B.
Principal Investigator: MAHADY, GAIL B PHD
Title: EVALUATION OF BOTANICALS FOR H. PYLORI INFECTIONS
Institution: UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL
Project Period: 2001/02/01-2002/12/31

DESCRIPTION (Applicant's Abstract): After more than a decade of research and controversy, it has been conclusively demonstrated that *Helicobacter pylori* is the main cause of peptic ulcer disease. High infection rates around the world pose serious health and economic problems. In the U. S. alone, 500,000 new cases, and 4 million recurrences are reported annually, at a cost of \$3 to \$4 billion dollars. Current therapies for *H. pylori* infections consist of combinations of antibiotics, and H2 blockers. However, due to the serious adverse reactions, patient compliance is low, leading to the development of antibiotic resistance. Thus, new approaches to the treatment *H. pylori* infections are urgently needed. For thousand of years traditional systems of medicine have successfully used botanicals (plant-based medicines) for the treatment of dyspepsia, gastritis and peptic ulcers. However, most of these botanicals have not been systematically screened for anti-*H. pylori* activity. This proposal describes an international, multidisciplinary approach to investigating botanical extracts for the treatment and prevention of *H. pylori* infections. The work is designed to generate sufficient preliminary data to serve as the basis of more definitive studies. The major goal of the project is to identify and standardize botanical extracts and combinations of extracts for the treatment of *H. pylori* infections. To accomplish this goal, the project involves (1) selection and procurement of botanicals to be tested (2) extraction of the source materials, (3) short-term in vitro and in vivo testing biological studies to determine activity and mechanistic information (4) in vivo evaluations to establish safety and efficacy, and (5) determination of the major chemical constituents for standardization of the active extracts. Over the two-year period, approximately 60 new botanicals will be selected and evaluated for antibacterial activity against *H. pylori*. The list of high priority botanicals for testing will be generated based on data analysis from the Napralertsm database, Medline, German Commission E Monographs and other data sources. The success of this method for plant selection is seen in our preliminary results where an initial testing of 25 identified botanicals led to 13 active extracts. In this project, the botanicals will be procured and extracts prepared. The botanical extracts will be subjected to in vitro bioassay protocols using 15 *H. pylori* strains. Active extracts will be utilized for in vivo studies. The long-term objectives are to develop safe and effective botanical extracts for the treatment and prevention of *H. pylori* infections.

Grant: 1R21AT000501-01
Program Director: WEST, NEAL B.
Principal Investigator: WILLIAMS, DAVID L
Title: Dietary supplementation with glucan enhances immune fn
Institution: EAST TENNESSEE STATE UNIVERSITY JOHNSON CITY, TN
Project Period: 2001/09/10-2003/08/31

DESCRIPTION (APPLICANT'S ABSTRACT): Glucans are natural product polymers of glucose, which are produced by fungi, certain bacteria and plants. Glucans have demonstrated the ability to stimulate immunity and increase resistance to infectious challenge following systemic administration, i.e., N, IP, IM or SQ. As natural products, glucans are found as fiber in the normal diet. Additionally, glucans have been identified as components of Chinese, Japanese and Indian traditional medicines. Recently, several dietary supplements have appeared on the market which are reported to contain glucan. The manufacturers have inferred from the scientific literature that dietary supplementation with glucans will have beneficial effects comparable to those observed with systemic administration. The development of a dietary supplement which could increase immunocompetence and enhance resistance to common infections would be of significant benefit to human health. However, the evidence that dietary supplementation with glucans will enhance immunity is limited and controversial and there is no evidence that these supplements will increase resistance to common infectious diseases. The objectives of this R21 exploratory research program are to determine whether dietary supplementation with glucan will modulate immune function and, more importantly, whether oral glucans will increase resistance to common infectious diseases. There are two specific aims. First, we will characterize the local and systemic immunomodulation associated with dietary supplementation of glucan. Second, we will determine whether oral administration of (1--3)-(3-D-glucans will enhance resistance to challenge with the respiratory pathogens, Streptococcus pneumoniae or murine adapted influenza A/PR8/34 HIM virus. At the successful conclusion of this research e will have established what, if any, effect dietary supplementation with glucan has on modulating immunocompetence arid whether oral glucans will increase resistance to infectious diseases.

Grant: 2R01CA059834-06A2
Program Director: IWAMOTO, KUMIKO
Principal Investigator: BRANCH, ROBERT A MD
PHARMACOLOGY:CLINICAL
THERAPEUTICS
Title: DRUG METABOLIZING ENZYMES-RISK FACTORS IN BLADDER CANCER
Institution: UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PA
PITTSBURGH
Project Period: 1994/07/01-2005/06/30

This molecular epidemiology proposal is to continue applying knowledge of pharmacogenomic implications of gene expression of individual drug metabolizing enzymes to assess their role as risk markers for bladder cancer. We propose to use measures of whole body activity for drug metabolizing enzymes using the Pittsburgh cocktail that comprises CYP1A2 (caffeine), CYP2C19 (S-mephenytoin), CYP2D6 (debrisoquine), CYP2E1 (chlorzoxazone) and CYP3A4 (dapsone), as well as mRNA concentrations for each of these CYP enzymes in leukocytes and genotypic identification of known polymorphisms of CYP metabolizing enzymes to include CYP2D6 and CYP2E1. We will assess acetylation using a phenotypic trait measure (dapsone), supplemented by genotyping as well as GSTMI, and GSSTI using genotyping. Our initial work has provided evidence that high activity for CYP2D6, low activity of CYP3A4, mutant alleles for acetylation and the null genotype for GSTMI are risk factors for bladder cancer, but to different extent for various forms of this cancer. We have also shown that high CYP2D6 activity is associated with mutations of the retinoblastoma (Rb) gene and low activity of CYP3A4 is independently associated with p53 mutations. Furthermore, different groups of risk factors relate to different mutational spectra of p53. We now propose to extend these observations. Our specific aim is to test the hypothesis that bladder cancer is comprised of a heterogeneous group of diseases in which different groups of associated risk factors relate to disease states that not only vary in etiology, but also in histopathological expression and natural history of the disease. This hypothesis will be evaluated in a case-control study of over 200 patients with incidence presentation of bladder cancer and over 200 controls matched for age, gender and ethnicity, in which environmental and constitutive variables will be related to the disease process. This study will involve a protocol that incorporates an exposure questionnaire, the Pittsburgh cocktail and blood sampling for mRNA quantitation and DNA genotyping. The disease process will be evaluated by clinical assessment and staging, identification of mutations of p53 and Rb genes, blinded histopathological review with grading and following the natural history for the disease. Collectively, these molecular epidemiology studies will improve our understanding of pathogenic mechanisms involved in different forms of bladder cancer and will expand our understanding of the regulation of the gene products that are responsible for drug metabolism in humans.

Grant: 2R01CA073041-05
Program Director: PELROY, RICHARD
Principal Investigator: DOETSCH, PAUL W PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: REVERSAL OF DNA DAMAGE BY ALTERNATIVE EXCISION REPAIR
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 1997/02/01-2006/01/31

The overall goal is to gain an understanding of the role of the alternative excision repair (AER) pathway in the reversal of DNA damage. A great deal of information was gained during the previous funding period which indicates that the AER pathway in the fission yeast *Schizosaccharomyces pombe* functions in the repair of a diverse group of DNA lesions ranging from UV bi-pyrimidine photoproducts to base mismatches. An emphasis will be placed on the functions of the AER initiating endonuclease Uve1p in *S. pombe*. In addition, further refinement of the known Uve1p-related pathways identified in the previous project will be pursued. The scope and focus of the present proposal is expanded considerably from the previous emphasis on UV damage. This is necessary in order to begin to define the multiple potential interrelationships between Uve1p and other DNA repair/processing pathways. Biochemical characterization of the components of the AER pathway and potentially related pathways will continue as well as studies aimed at determining the range of organisms which utilize Uve1p-like proteins for DNA repair.

Grant: 1R01CA082766-01A2
Program Director: YOVANDICH, JASON
Principal Investigator: PENNELL, CHRISTOPHER A BS
Title: GENETICALLY OPTIMIZED IMMUNOTOXINS FOR LEUKEMIA THERAPY
Institution: UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN
Project Period: 2001/01/30-2003/12/31

DESCRIPTION: (Applicant's Abstract) The applicant's long-term goal is to develop novel, targeted therapeutics for the treatment of human T cell malignancies. Immunotoxins (ITs) are a class of therapeutic agents with a high degree of specificity and a unique mechanism of action. An IT is a hybrid molecule consisting of a targeting moiety linked to a toxin. The targeting moiety selectively binds to a tumor cell and targets it for death via the attached toxin. Generally, ITs are specifically potent against cancer cells in vitro and in animal models of human malignancies. However, ITs are limited clinically by immunogenicity, toxicity, and/or instability. A clinical grade IT called DA7 was synthesized at the University of Minnesota by biochemically linking deglycosylated ricin toxin A chain (dgRTA) to a monoclonal antibody specific for the T cell-associated antigen CD7. A Phase I clinical study of DA7 revealed that its efficacy was primarily limited by instability and nonspecific vascular toxicity. Despite these limitations, DA7 achieved objective clinical responses at the maximal tolerated dose. If the instability and vascular toxicity of DA7 were reduced, then the applicant contends that DA7 could find a therapeutic niche in the treatment of refractory T cell disease, or as an adjuvant to surgery or chemoradiotherapy. The objective of this revised application is to use genetic engineering to enlarge the 'therapeutic window' of DA7 by increasing its stability and decreasing its toxicity. The first specific aim focuses on the construction and testing of recombinant ITs containing a derivative of Diphtheria toxin (DT) linked to CD7-specific single chain Fv (sFv) fragments. DT will be used initially since it is a component of the only FDA-approved immunotoxin. Modifications of the sFv structure will be made to enhance stability. Novel approaches for the high-level expression of soluble fusion toxins, and for the direct visualization of IT-mediated tumor cell killing in vivo, are included in this aim. The second specific aim focuses on decreasing toxicity (and immunogenicity) by linking the most stable sFv structure identified in Specific Aim 1 to human RNAses. The RNAses will be engineered to be both resistant to RNase inhibitors and to be optimally cytotoxic once internalized. All immunotoxins proposed in Specific Aim 1 and Specific Aim 2 will be tested for their stability, relative affinity, pharmacokinetics, toxicity, and anti-tumor activity. These experiments will allow the applicant to determine more precisely the relationships between stability, toxicity, and efficacy. His ultimate goal is to return to the clinic with a more potent version of DA7.

Grant: 1R01CA085777-01A1
Program Director: OKANO, PAUL
Principal Investigator: ROSENBERG, SUSAN M
Title: DNA REPAIR AND REPLICATION RE START IN VIVO
Institution: BAYLOR COLLEGE OF MEDICINE HOUSTON, TX
Project Period: 2001/01/17-2005/12/31

Genetic instability is a primary cellular event leading to cancers, tumor progression, and resistance to chemotherapeutic drugs. Chromosomal changes involved include somatic recombination between homologous chromosomes, promoting loss of heterozygosity, translocations between non-homologous chromosomes, gene amplification, and other DNA rearrangements in addition to mutation. These various forms of genetic instability are provoked by DNA repair events. Many of the human proteins implicated in genetic instability have homologs in eubacteria which appear to function similarly, many of which participate in DNA repair including repair of double-strand breaks (DSBs) and single-strand gaps. Agents that cause the lesions that require repair are primary carcinogens, and human genetic diseases in which homologs of the bacterial repair proteins are altered display cancer-proneness. Although genetic rearrangements and DNA replication have been thought of as separate events, and have been studied separately, many lines of recent evidence from several organisms now imply a profound connection--the promotion of DNA replication by double-strand DNA break-repair (DSBR). In only one cellular (non-viral) system has this connection been firmly and directly demonstrated: DSBR in *E. coli* occurs roughly half the time as a replication-promoting event, and the other half via recombination without replication. In this proposal the molecular mechanism of DSBR in the *E. coli* model system is investigated with the goals of elucidating structures of DNA intermediates in and products of the process, identifying all of the relevant proteins involved, and understanding control of replicative versus non-replicative mechanisms. This information will be related to genetic instability in cancer, and, in some cases, the human orthologs of *E. coli* repair proteins will also be examined. Because direct physical analysis of DNA, as well as sophisticated genetic tools are available in this model system, the level of detail of molecular mechanism to be obtained is unparalleled in other organisms. Because the proteins so far identified are homologous with human cancer proteins (e.g. Blm, Wrm, hRad51 which functions with Brca proteins in DSBR), and their functions are conserved, the information is directly applicable to the mechanisms of genetic instability that cause, promote, and make drug-resistant, human cancers.

Grant: 1R01CA086079-01A1
Program Director: DUBOIS, RONALD J.
Principal Investigator: THEODORAKIS, EMMANUEL A PHD
Title: Total Synthesis of Bioactive Natural Products
Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA
Project Period: 2001/03/01-2006/02/28

DESCRIPTION: (Applicant's Description) The first objective of this proposal is to study a novel methodology that allows access to substituted quinones and evaluate its scope and limitations during the synthesis of bioactive natural products, such as ilimaquinone avarone and avarol. The second objective of this proposal is the synthesis of norrisolide, a natural product for which in vivo studies have demonstrated that it causes irreversible vesiculation of the Golgi complex. A third objective of our research is the synthesis of borrelidin, an unusual macrolide with a very potent profile as an angiogenesis inhibitor.

Grant:	1R01CA092229-01	
Program Director:	DASCHNER, PHILLIP J	
Principal Investigator:	FALKOW, STANLEY	PHD BIOLOGY NEC:BIOL NEC-UNSPEC
Title:	Helicobacter Host Interactions in Animal Models	
Institution:	STANFORD UNIVERSITY	STANFORD, CA
Project Period:	2001/07/01-2005/06/30	

DESCRIPTION (provided by applicant): Helicobacter pylori infection is causally associated with gastritis and peptic ulcer, as well as two gastric malignancies, gastric carcinoma and B-cell-mucosa-associated lymphoid tissue (MALT) lymphoma. Our proposed research focuses on the application of genetic and molecular tools to manipulate the H. pylori chromosome and the use of DNA microarray technology to monitor both the host and the pathogen in H. pylori animal models of infection and disease. Specifically, we propose to examine the H. pylori infection of mice and Mongolian gerbil. While none of the cell culture models or the animal models we propose to use can fully reflect what is seen in humans, the mouse model of infection can be used to productively investigate how H. pylori colonizes the stomach. We wish to follow long-term infection of the mouse and the host cell response to long-term H. pylori carriage measured by transcriptional profile changes as compared to uninfected littermates. Also, the mouse infection model is useful to study one form of malignancy caused by H. pylori, MALT lymphoma, and we propose to study this feature of long-term H. pylori murine infection by both bacterial transcription profiling and by the use of a mouse DNA microarray to follow the host response and changes that occur in the malignant transformation. We also propose to identify bacterial genes essential for gastric colonization and persistence in the stomach using a method developed in our laboratory called MicroArray Transposon Tagging (MATT) strategy. H. pylori infection reflects a particularly intriguing example of a host-pathogen interaction. The microbe serves as a tool to understand host cell biology and malignancy. The response of the bacterium to the host cell environment allows us to understand the essence of bacterial pathogenicity.

Grant: 1R01CA093405-01
Program Director: DASCHNER, PHILLIP J
Principal Investigator: WANG, TIMOTHY C MD MEDICINE
Title: Mouse Models of Gastric Cancer
Institution: UNIV OF MASSACHUSETTS MED SCH WORCESTER, MA
WORCESTER
Project Period: 2001/05/01-2006/04/30

DESCRIPTION: (Adapted from the investigator's abstract) *Helicobacter pylori* infection has been strongly linked to both hypergastrinemia and gastric cancer, but the role of elevated serum gastrin levels in progression to malignancy has not been well studied. Previous investigations have suggested that parietal cell loss or gastric atrophy represents a key preneoplastic precursor. Our group has developed a hypergastrinemic transgenic (insulin-gastrin or INS-GAS) mouse model that shows progression over time to gastric atrophy, intestinal metaplasia, dysplasia and gastric cancer. Further analyses of our INS-GAS mouse model, as well as studies in gastrin deficient (GAS-KO) mice, suggest that chronic elevations of amidated gas trin (G- 17) can lead to parietal cell decline, which can be prevented by infusions of incompletely processed glycine-extended gastrin (G-gly). Gastrin may also promote the development of cancer through induction of cyclooxygenase-2 (COX-2), resulting in increased proliferation and upregulation of VEGF. *Helicobacterfelis* infection of INS-GAS mice leads to a marked acceleration of gastric cancer and early mortality, suggesting a strong synergistic interaction between hypergastrinemia and *Helicobacter* infection. We propose to explore further the role of gastrin in gastric carcinogenesis. (1) A possible interaction between hypergastrinemia and p53 mutations will be investigated. Alterations in the p53 gene will be investigated in neoplastic lesions, and INS-GAS mice will be crossed with p53 null mice and the response to *Helicobacter* infection tested. (2). Possible downstream targets (COX2 and VEGF) in gastrin/*Helicobacter*-dependent gastric cancer will be studied. Selective COX-2 antagonists will be administered to *Helicobacter*-infected INS-GAS mice, and INS-GAS mice will be crossed to VEGF-GFP transgenic mice to assess VEGF gene expression during cancer progression. (3). The importance of the parietal cell CCK-B/gastrin receptor and Gq signaling pathways will be determined. Highly specific CCK-B receptor antagonists will be administered, and a constitutively active Gq-coupled CCK-B receptor targeted to the parietal cell in transgenic mice. (4). The possible protective effective of glycine-extended gastrin, (G-gly) in the prevention of atrophy/cancer will be studied. Double transgenic mice (INS-GAS x G-gly) or INS-GAS mice receiving infusions of 0-gly will be infected with *Helicobacter* and progression to atrophy and cancer analyzed. Overall, these studies will explore the mechanisms by which gastrin may influence the parietal cell and susceptibility to gastric neoplasia.

Grant: 1R15CA088893-01
Program Director: GROTZINGER, KAREN R
Principal Investigator: WRIGHT, BARBARA E PHD
Title: COULD TRANSCRIPTION INDUCED MUTATIONS CAUSE CANCER?
Institution: UNIVERSITY OF MONTANA MISSOULA, MT
Project Period: 2001/04/09-2004/03/31

DESCRIPTION: (Adapted from the investigator's abstract) There may be a connection between malnutrition, transcription, mutations and cancer. In mammals, a number of genes are derepressed in response to caloric restrictions, and amino acid limitation can also induce gene expression, which increases mutation rates. In a model system, the effect of transcription (activated by guanosine tetraphosphate, or ppGpp) on mutation rates and mutations in the leu operon of *E. coli* will be examined. Specific aims are to determine the: A. Kinetics of mRNA turnover during logarithmic growth and during leucine starvation. B. Effect of ppGpp on the kinetics of transcription initiation in leuB and pyrD. C. Effect of antisense RNA and DNA on leuB. The proposed investigations have implications for understanding mechanisms of mutations and cancer. It is estimated that the majority of cancers are caused by environmental (including nutritional) conditions. Data indicate that transcription can enhance the rate of both point mutations and frameshifts, suggesting that genetic derepression may be a major cause of background mutations. If so, this mechanism provides the critical link to metabolic activities evoked by malnutrition, and could play a role in directing which mutations occur. Basic research on the vulnerability of single-stranded DNA to mutation and repair may well reveal insights into the mechanisms of tumorigenesis, and thus aid in the prevention or treatment of cancer.

Grant: 1R21CA091086-01
Program Director: ARYA, SURESH
Principal Investigator: HU, GUO-FU PHD
Title: Inhibition of Angiogenin enhanced rRNA Transcription
Institution: HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA
Project Period: 2001/03/01-2003/02/28

Sustained cell growth, a hallmark of cancer, requires continuous protein synthesis that depends on an adequate supply of ribosomes. Transcription of ribosomal RNA is a rate-limiting step in ribosome biosynthesis. Inhibition of ribosomal RNA transcription is therefore an effective means to decrease ribosomal biogenesis thereby inhibiting cell growth. Angiogenin, whose concentration is elevated in many cancers, has recently been found by our group to undergo nuclear translocation in actively proliferating cells where it binds to the promoter region of the ribosomal RNA gene and stimulates rRNA transcription. The nuclear function of angiogenin is confined to proliferating cells that would include cancer cells and some normal cells only under certain circumstances such as during development and wound healing. The purpose of this exploratory/development grant application is to determine whether or not angiogenin is involved in transcription of ribosomal RNA in cancer cells and whether or not inhibition of angiogenin-enhanced rRNA synthesis can serve as a new molecular target for cancer drug development. Experiments are designed (1) to examine the nuclear translocation profile of angiogenin in cancer cells thereby determining the differential behavior of angiogenin in cancer and normal cells; (2) to investigate angiogenin-enhanced rRNA synthesis in cancer cells and its relationship with nuclear accumulation of endogenous as well as exogenous angiogenin; and (3) to inhibit nuclear translocation of angiogenin in cancer cells and determine the effect of this inhibition on rRNA synthesis, growth rate, and cell proliferation. Results obtained would determine whether it is feasible to develop anticancer drugs based on the intervention of the nuclear function of angiogenin.

Grant: 1R21CA091116-01
Program Director: HALLOCK, YALI
Principal Investigator: DAVISSON, VINCENT J PHD ORGANIC CHEMISTR
Title: Enzyme Targets, Apoptosis and Purine Metabolism
Institution: PURDUE UNIVERSITY WEST LAFAYETTE WEST LAFAYETTE, IN
Project Period: 2001/04/01-2003/03/31

DESCRIPTION: (provided by applicant) Many drugs from nature present complex molecular architectures that result in varied degrees of specific interactions with cellular targets. The rational selection and implementation of new cancer therapies would be improved if a basis for assessing all the drug target interactions were available. This proposal will address the importance of multiple drug targets for the ansamycin class of antitumor agents. Our efforts will focus on the molecular target(s) for the experimental breast cancer drug 17-allylamino-17-demethoxygeldanamycin (17-AAG). The benzoquinoid ansamycins geldanamycin (GA) and herbimycin A and the nonapolyketide radicicol have been investigated for their antiproliferative activity showing both cytostatic and cytotoxic activities. Recent studies predict the mode of action for the cytotoxicity is associated with inhibition of the cellular chaperone Hsp90 or its homolog Grp94. This proposal challenges the assumptions that Hsp90 is a primary mode for induction of tumor cell death by GA. We have recently identified a high-affinity binding site for the drug geldanamycin on an enzyme in the de novo purine biosynthetic pathway called ADE2. A thorough analysis of this interaction in tumor cells and its consequences for drug action will provide the scientific basis for using this information in future applications of this drug class. A detailed biochemical characterization of the human ADE2 protein from tumor cells and an investigation of the molecular details dictating its inhibition by GA are proposed. These studies will assess if ADE2 is a suitable target for cancer chemotherapy and if purine depletion is a critical step in the induction of apoptosis. The studies constitute a biorational approach toward a new perspective on targets for anticancer drugs in de novo purine metabolism. A mechanistic understanding of the biochemical role and regulation of the human ADE2 protein in tumor cells will be a fundamental basis for this analysis.

Grant: 1R01DA013256-01A1
Program Director: THADANI, PUSHPA
Principal Investigator: KNUEPFER, MARK M PHD PHARMACOLOGY,
OTHER
Title: Sympathetic Regulation of Endotoxemia in Drug Abuse
Institution: ST. LOUIS UNIVERSITY ST. LOUIS, MO
Project Period: 2001/04/01-2006/02/28

DESCRIPTION: Host defense responses to endotoxemia vary in individuals. Cocaine use further compromises responses to microbial infections in some humans, although the factors responsible for individual predisposition to septic shock in the context of drug abuse is particularly complex and poorly understood. It appears that multiple factors interact to determine individual responsiveness including autonomic regulation of the acute inflammatory responses. This proposal is based on the premise that cocaine will enhance the predisposition to septic shock more readily in some individuals than others. We propose that divergent vascular responsivity to cocaine plays a pivotal role in the induction of cardiovascular dysfunction, including the septic shock syndrome from Gram-negative infectious agents. The effects of the sympathomimetic, cocaine, are potentially on both cardiovascular derangements and on pathogen-induced cytokine gene expression, thereby reducing survival during endotoxemia. In our model of cocaine-induced cardiomyopathies and hypertension in conscious rats, cocaine evokes substantial increases in systemic vascular resistance (SysVR) and renal sympathetic nerve activity concurrent with reductions in cardiac output (CO) in animals designated vascular responders. In contrast, mixed responders have smaller increases in SysVR and increases in CO. Our evidence suggests that differences between vascular and mixed responders depend on divergent CNS-mediated sympathetic responses to cocaine. We hypothesize that the excessive sympathetic responsiveness to cocaine and endotoxemia noted in vascular responders results in life-threatening shock due to either a greater loss in receptor sensitivity or to enhanced expression of pro-inflammatory cytokines. This proposal will reveal the causes of alterations in the prognosis of endotoxemia after acute and chronic cocaine use. First, we will verify that acute cocaine pretreatment differentially affects cardiovascular responses, cytokine expression and lethality in rats exposed to Gram-negative endotoxemia. We will utilize lipopolysaccharide (LPS) as a self-limiting model of Gram-negative inflammation. Second, we will determine the contribution of peripheral adrenergic receptors and the sympathetic nervous system that are responsible for variations in individual sensitivity to LPS-induced endotoxemia in animals exposed to cocaine. Third, we will determine the role of specific peripheral autacoids in mediating variable hemodynamic and cytokine responses to LPS. We will assess proinflammatory cytokine synthesis and levels in the plasma, liver, spleen and lungs and block the actions of IL-1B and TNF-alpha. Fourth, we will examine the effects of chronic exposure to cocaine on patterns of autonomic regulation and cytokine expression to acute endotoxemia. Finally, we will study the potential contribution of autonomic and cytokine responsiveness to susceptibility to endotoxemia in rats exposed to repeated cocaine. These studies will provide novel insights into the pathogenesis of cardiovascular dysfunction during infection-related inflammation and clarify the role of the sympathetic nervous system in modulating vascular tone and cytokine responsivity. This

information will contribute to our understanding of predisposing factors to the incidence and severity of septic shock in humans and the mechanisms by which cocaine use affects this process.

Includes RPGs (Research Project Grants)
Excludes Clinical Trials

Grant: 1R21DA014942-01
Program Director: HILLERY, PAUL
Principal Investigator: JAVITCH, JONATHAN A MD
Title: ARCHAEOAL & BACTERIAL HOMOLOGS OF DOPAMINE TRANSPORTER
Institution: COLUMBIA UNIVERSITY HEALTH SCIENCES NEW YORK, NY
Project Period: 2001/09/30-2003/09/29

DESCRIPTION: (provided by the applicant) Following the release of dopamine, its concentration in and around the synapse is rapidly reduced by the dopamine transporter (DAT), the major molecular target of cocaine and related psychostimulants. DAT requires extracellular sodium and chloride and couples the translocation of dopamine to the movement of these ions down their concentration gradients. Despite the cloning of DAT and related neurotransmitter transporters, its molecular structure and transport mechanisms are poorly understood. Progress in this area has been hampered by the lack of sufficient functional, purified protein and the inability to develop high-level expression systems for these proteins. Bacterial membrane proteins are generally more amenable to structural analysis and high-level expression than are their eukaryotic counterparts. We have recently identified an entire family of proteins in archaea and in bacteria that are homologous to DAT. Currently 39 proteins from 21 different organisms appear to fall into this family. None of these transporter proteins have been studied, and their substrates are unknown. In this CEBRA application, we propose to intensively explore the properties of a subset of these DAT archaeal and bacterial homologs in order to assess their suitability for use in further direct and indirect structural studies. Thus we propose the following specific aims: 1) To clone the 9 sodium- and chloride-dependent transporters from archaea and bacteria that are most similar to DAT. 2) To express these proteins heterologously in *E. coli*, and to assess and optimize levels of plasma membrane expression as well as solubilization and purification conditions. 3) To express a candidate transporter(s), which based on studies in Aim 2 is suitable for further structural analysis, in *Xenopus laevis* oocytes to facilitate studies using electrophysiological techniques, focusing initially on sodium-dependent transient currents to determine whether the transporter(s) is inserted into the membrane and is functional. 4) To identify the substrates and/or non-substrate inhibitors of this transporter(s) by the measurement of substrate-induced currents. At the end of the period of support we will be in a position to choose a limited number of these archaeal and bacterial transporters for use in crystallization trials as a preliminary step towards obtaining a high-resolution structure. Moreover, we will also be poised to pursue biochemical and biophysical methods to acquire functional data and indirect structural information about these transporters. The resulting information will likely revolutionize our structural understanding of the function of DAT and related human neurotransmitter transporters, such as the serotonin and norepinephrine transporters that are targets for antidepressant drugs and cocaine, in a way that is only a distant prospect through continued work on the eukaryotic transporters alone.

Grant: 1R01DC004555-01A1
Program Director: FREEMAN, NANCY
Principal Investigator: STEYGER, PETER S PHD
Title: HAIR CELL RESPONSES TO OTOTOXIC DRUGS
Institution: OREGON HEALTH & SCIENCE UNIVERSITY PORTLAND, OR
Project Period: 2001/09/01-2006/07/31

Although clinical use of aminoglycoside antibiotics is essential against life-threatening bacterial infections, there are serious ototoxic and nephrotoxic side-effects in 4-14 percent of all aminoglycoside prescriptions (4 million annually). Ototoxicity causes sensory hair cell death, hearing loss, and vestibular disorders, leading to physical, mental, educational, and language difficulties in patients. The overall aim of our studies is to identify, and then prevent the cellular mechanisms that initiate ototoxic drug-induced hair cell death. The long-term goal of this project is to develop interventional strategies that will allow future clinicians to use aminoglycosides without serious side effects. Gentamicin and forskolin are two unrelated ototoxic drugs that depolarize mitochondria, and increase production of reactive oxygen species and calcium levels in hair cells. Each of these toxic sequelae is a powerful trigger for inducing hair cell death. These common cellular responses to toxicity suggest that unrelated ototoxic drugs trigger a common pathway that lead to hair cell death. Therefore, we will use explants of bullfrog saccular hair cells to: (1) Identify the acute effects of gentamicin and forskolin in hair cells, and determine if they trigger these toxic sequelae by similar or differing mechanisms (2) Determine if inhibitors of drug-uptake can prevent toxic sequelae in hair cells (3) Determine if effective inhibitors of drug-uptake and toxic sequelae enhance hair cell survival during ototoxic drug treatment These studies provide a direct link between the molecular and biochemical studies of ototoxic drugs and the morphological analysis of fixed tissues after drug treatment. This knowledge will advance development of clinical strategies to prevent drug-induced hair cell death, and preserve inner ear function during this critical pharmaceutical therapy.

Grant: 1R01DC004976-01
Program Director: DAVIS, BARRY
Principal Investigator: MCGHEE, JERRY R PHD IMMUNOLOGY, OTHER
Title: Ab5 Toxins Nasal Adjuvant Target the Olfactory Bulbs/CNS
Institution: UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL
BIRMINGHAM
Project Period: 2001/04/01-2005/03/31

DESCRIPTION: (Adapted from Applicant's Abstract) The intranasal route of vaccination has become the predominant one used in mucosal immunology in order to induce both systemic and mucosal T cell and antibody (Ab) responses. Nasal application of vaccines with adjuvants is easy to perform, requires 10 - 20 fold less material than does oral immunization and the vaccine and adjuvant are not subjected to degradative enzymes as they are in the GI tract. Despite these advantages, it is often not appreciated that the nasal region is extensively innervated by the olfactory bulb, which is directly connected via olfactory nerves to the olfactory epithelium. Both native enterotoxins like cholera and E. coli labile toxin (CT and LT), and nontoxic derivatives have pentameric B subunits, which bind to GM1 (CT) and to GM2 and asialo GM1 (LT) which are expressed by the olfactory epithelium and the associated olfactory nerves. It was shown that either CT or anti-GM1 Abs induced CNS lesions when injected into the lumbosacral subarachnoid space. This toxicity is consistent with expression of GM1 by astroglia and neurons of the CNS and by microglial cells of the cerebellar cortex. We have therefore postulated that intranasal application of nCT or nontoxic mCTs, which have intact (pentameric) CT-B, could enter the CNS through GM1 binding to olfactory epithelium and nerves with subsequent transport into the olfactory bulb. This would postulate that retrograde axonal transport would bypass the blood / brain barrier. Further, either nCT or mCT could potentially damage the CNS and even induce CNS uptake of co-nasally delivered vaccine proteins. The first Specific Aim to test this hypothesis will actually examine the trafficking of nCT or mCTs in olfactory epithelium and the CNS and whether these molecules induce neuropathologic changes. The second Specific Aim will determine if nCT or mCTs also induce uptake and trafficking of co-administered vaccine proteins into the nasal olfactory epithelium, nerves, and bulb. The third Specific Aim will determine potential toxicity of nCT and mCTs for cultured astrocytes and microglial cells, including inflammatory cytokine production. The fourth Specific Aim will extend this analysis of toxic, inflammatory and apoptotic responses to the nerve cells themselves in the olfactory bulb when exposed to nCT or mCTs. The final Specific Aim will assess long-term effects of CT-B binding to GM1 on neural cells, including the induction of autoimmune anti-GM1 Abs. These proposed studies will provide essential new information to determine potential side effects of CT-B derivatives as nasal adjuvants, and point to ways where these toxic effects can be avoided.

Grant: 1R01DC005025-01
Program Director: WATSON, BRACIE
Principal Investigator: LIM, DAVID J MD
OTORHINOLARYN:OTOLOG
Title: Innate Immunity in Otitis Media Pathogenesis
Institution: HOUSE EAR INSTITUTE LOS ANGELES, CA
Project Period: 2001/09/10-2004/08/31

DESCRIPTION (provided by applicant): Otitis media (OM) is one of the major causes of morbidity and the most common cause of hearing loss in children. Eighty percent of the children born each year experience at least one episode of OM by their third birthday, and one in three have repeated bouts of the disease. OM has an annual cost of \$5 billion and accounts for an estimated 31 million annual visits to the doctor's office. Due to the rapid worldwide increase in antibiotic resistance among OM pathogens there is now an urgent need to develop new and innovative non-antibiotic approaches to prevent and manage this disease. To this end, it is imperative to understand how the molecules of the innate (natural) immune system function and protect the tubotympanum before the activation of adaptive immunity. We hypothesize that the tubotympanum is protected by an highly effective innate immune system, including the secreted antimicrobial innate immune molecules, such as lysozyme, lactoferrin, B-defensins, and surfactant proteins A and D (SP-A and SP-D). We further hypothesize that innate immune molecules can act synergistically to maximize their anti-microbial activities against pathogens and that their immaturity or poor function is a risk factor for OM. Our long-term goal is to elucidate the role of innate immunity in OM pathogenesis and to develop novel preventative and therapeutic measures. Towards our objective, we will: 1) determine the individual and synergistic bacteriostatic and bactericidal activities of the innate immune molecules against the major OM pathogens - nontypable *H. influenzae* (NTHi), *S. pneumoniae* and *M. catarrhalis*; 2) determine the in vivo time course of changes in the expression of innate immune molecules in the rat middle ear in response to inactivated NTHi; 3) identify the intracellular signal transduction cascade(s) that result in the activation of the innate immune molecules; 4) determine the role of the anti-microbial surfactant proteins, SP-A and SP-D, in OM using knock out mice for these molecules.

Date Run: 04/26/05

NIH Extramural Support in Bacteriology Research

Grant: 1R55DC004583-01A1

Program Director: WATSON, BRACIE

Principal Investigator: GOLDSTEIN, RICHARD N

PHD BIOLOGY NEC:BIOL

NEC-UNSPEC

Title: MICROBIAL POPULATION STRATEGY FOR VACCINE DEVELOPMENT

Institution: BOSTON MEDICAL CENTER

BOSTON, MA

Project Period: 2001/02/01-2003/01/31

Abstract Text Not Available

Grant: 2P01DE011549-06
Program Director: MANGAN, DENNIS F.
Principal Investigator: BOWEN, WILLIAM H
Title: ENVIRONMENTAL INFLUENCES AND DENTAL CARIES
Institution: UNIVERSITY OF ROCHESTER ROCHESTER, NY
Project Period: 1995/09/01-2005/12/31

Despite significant reduction in the prevalence of dental caries in some segments of the population it remains a major public health problem, particularly for those least able to bear the burden. The decline in prevalence has generally been attributed to increased exposure to fluoride through a variety of routes. Prior to and during the increased use of fluorides ingestion with food preservatives was also increasing by over 25 fold. Food preservatives have properties in common with fluoride in that they behave as weak acids they are protonated at low pH values, can diffuse into cells, reduce acid tolerance and acid adaptation by mutants streptococci. Recognizing the importance of saliva on the plaque environment, we will examine the relationship of mammalian and non- mammalian components of whole saliva samples collected over a three- year period, to the incidence of dental caries in the same group of subjects. We believe this proposal is highly novel and offers the possibility of developing approaches to enhance the diagnosis of caries and methods for its prevention.

Grant: 2R01DE004733-23
Program Director: MANGAN, DENNIS F.
Principal Investigator: TAUBMAN, MARTIN A
Title: Synthetic Peptide Vaccines for Dental Caries
Institution: FORSYTH INSTITUTE BOSTON, MA
Project Period: 1996/05/01-2006/06/30

Our overall aim is to develop immunization constructs, vectors, and strategies which have the best chance of inducing protective immune responses to mutans streptococcal infections in susceptible pediatric populations. We have previously identified several glucosyltransferases (GTF) enzyme sequences which can induce immune response in rodents which will alter GTF activity and reduce subsequent dental caries. To enhance the induction of protective immune responses we will first identify sequences from glucosyltransferase (GTF) enzymes from *S. mutans* and *S. sobrinus* associated with potent Class II MHC binding in humans. Peptides based on these and on novel GTF epitopes, recently shown to influence catalytic activity and transitional state stability of the enzyme (activity associated) will be synthesized and evaluated for immunogenicity and protective effect. These epitopes, together with other functionally significant peptides derived from the catalytic and glucans binding domains of GTF previously shown to induce protective responses, will be incorporated into conjugate vaccines with tetanus toxoid (TT). Intranasal routes for mucosal immunization with TT- GTF peptide conjugate vaccine will be investigated for the ability to induce protective levels of immunity in the oral cavity in the well established rodent model of experimental dental caries. Since the intranasal route may be contraindicated in children with upper respiratory conditions such as asthma, the colo-rectal route of administration will also be explored using constructs combined with or without mucosal adjuvants such detoxified mutant *E. coli* enterotoxin (LT) or unmethylated CpG oligodinucleotides. The protective effect of systemic immunization with TT-GTF peptide conjugate vaccine studies will also be investigated as an approach to minimizing the frequency of visits required for childhood immunizations, since evidence suggests that this route of immunization can induce salivary IgA antibody in young children. Various of the most effective peptide(s) as determined by TT-GTF peptide conjugate vaccine studies will be expressed recombinantly in attenuated *Salmonella typhi* vectors which can target these epitopes to appropriate mucosal inductive sites. Caries protection will be evaluated after intranasal immunization with these attenuated *Salmonella* vectors. Our goal is to design a vaccine that contains a combination of immunologically potent and functionally relevant epitopes, in formats, by routes, and with adjuvants that result in sustained levels of protection from dental caries and that will be acceptable for human use.

Grant: 2R01DE006014-19
Program Director: MANGAN, DENNIS F.
Principal Investigator: SHENKER, BRUCE J PHD
MICROBIOLOGY:IMMUNOLOGY
Title: Bacteria and Lymphocyte Suppression in Periodontitis
Institution: UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA
Project Period: 1982/08/01-2006/02/28

Over the past several years, significant progress has been made in understanding of the etiology and pathogenesis of periodontal diseases. Nevertheless, the nature and contribution of the immune system to these disorders remain unclear. The application presents the hypothesis that the immune system plays a primary role to minimize and/or prevent infection. Furthermore, the application posits that immunoregulatory abnormalities contribute to the pathogenesis of and susceptibility to periodontal disease. In this regard, the preliminary investigations have demonstrated that several periodontal pathogens produce factors capable of impairing human T- and B-cell function; these include *Actinobacillus actinomycetemcomitans*, *Fusobacterium nucleatum* and *Treponema denticola*. The fundamental hypothesis of the planned studies is that periodontal pathogens produce immunosuppressive proteins (ISPS) that mediate local and/or systemic immunosuppression, thereby enhancing their own virulence and/or that of other opportunistic microorganisms. The current plan is to focus this investigation on the *A. actinomycetemcomitans* ISP, recently shown to induce human lymphocytes to irreversibly arrest in the G2 phase of the cell cycle. Moreover, the applicants have determined that this ISP is the product of the *cdtB* gene, one of three genes encoding the family of cytolethal distending toxins. The objectives of this application are to define the cascade of events responsible for CdtB-induced G2 arrest and to determine the relationship between structure and function of this ISP. The study is composed of four Specific Aims: 1) to determine the underlying molecular mechanism(s) responsible for the failure of CdtB-treated T-cells to de-phosphorylate and, thereby activate, the cyclin dependent kinase (cdk), *cdkl*; 2) to determine if a receptor is involved in the binding of CdtB and/or the holo-Cdt toxin to the surface of T-cells; 3) to determine if CdtB is part of a hetero-oligomer that forms a holo-Cdt toxin; and 4) to determine if conserved motifs that exist in all known CdtB polypeptides represent functional domains of the protein.

Grant: 2R01DE009018-11
Program Director: ZHANG, GUO HE
Principal Investigator: STASHENKO, PHILIP P DMD DENTISTRY
Title: Immunomodulation of Inflammatory Bone Resorption
Institution: FORSYTH INSTITUTE BOSTON, MA
Project Period: 1989/03/15-2006/06/30

Optimal host resistance to microbial pathogens requires the selective activation of a particular cellular or humoral immune response. Delayed-type hypersensitivity responses mediated by Th1 cells are required to combat infection with obligate intracellular organisms, whereas Th2 cells are beneficial in infections with extracellular organisms, including oral pathogens. New immunization strategies developed by us and others show great promise in directing responses toward either a Th1 or Th2 phenotype. Infections of the dental pulp result in pulpal necrosis and the resorption of periapical bone. During the prior grant period, we found that pulpal infection elicits predominantly Th1-type pro-inflammatory responses, and that periapical bone resorption is dramatically inhibited by the endogenously expressed anti-inflammatory Th2 cytokine IL-10. In the proposed studies, we will test the hypothesis that these novel immunization strategies can be used to skew responses against the model organism *P.gingivalis* toward a Th2 phenotype, resulting in increased IL-10 expression and reduced periapical bone resorption. In Aim 1, mouse strains that are genetically susceptible or resistant to *P. gingivalis*-induced periapical bone resorption will be identified. T cell lines from a resistant strain will be used to identify *P.gingivalis* antigens that preferentially stimulate IL-10 responses, using expression cloning (Aim 2). Aim 3 will determine if immunization with *P. gingivalis* antigens can inhibit periapical bone resorption in vivo. The mechanism(s) by which *P.gingivalis* antigens induce IL-10 responses will be characterized in Aim 4. The goal of these studies is to devise new methods for immunomodulating proinflammatory pathways and bone resorption via preferential induction of IL-10.

Grant: 2R01DE009081-11

Program Director: MANGAN, DENNIS F.

Principal Investigator: MICHALEK, SUZANNE M PHD
MICROBIOLOGY:IMMUNOLOGY

Title: Genetically Engineered Oral Vaccines & Caries Immunity

Institution: UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL
BIRMINGHAM

Project Period: 1996/02/01-2006/03/31

DESCRIPTION: Studies aimed at inducing immunity against infectious diseases, including Dental caries, have provided valuable information on microbial antigens important in inducing protective responses, the role of the mucosal immune system and IgA antibodies in defense against infections involving surfaces bathed by external secretions, and mechanisms involved in the induction of immune responses. The overall goal of this project is to define mechanisms by which mucosal vaccines consisting of recombinant, avirulent *Salmonella* strains expressing cloned genes of mutans streptococci, with and without adjuvant induce specific immune responses to the cloned antigen, which provide long-term protection. Specifically these studies will: 1) Determine the effect of persistence of the *Salmonella* vaccine strain and the amount of the expressed cloned antigens of mutans streptococci on the induction, nature and memory of immune response. Levels and isotype of antibodies to cloned antigens in serum and external secretions of animals immunized by the oral or intranasal (IN) route with *Salmonella* vaccines which persist for short or long times in the host, and which produce various amounts of cloned antigen will be measured by ELISA to determine the effect of these characteristics on the induction of mucosal immune responses. Protection will be assessed in an experimental model. The effect of *Salmonella* on the immune response to the cloned proteins will be characterized by measuring antigen-specific proliferation, cytokine production by ELISA and ELISPOT assay, and expression of co-stimulatory molecules by FACS in cell preparations from systemic and mucosal tissues. 2) Determine the effect of mucosal adjuvants on modulating host responses to recombinant antigens of mutans streptococci. Levels and isotype of antibodies to cloned antigens of mutans streptococci in serum and secretions of animals immunized by the oral or IN route with chimeric protein consisting of cloned antigens genetically linked to the B subunit of cholera toxin (CTB) or *Salmonella* vector vaccine expressing various amounts of chimeric proteins +/- free CTB will be measured by ELISA. The effect of the *Salmonella* on the adjuvant properties of CTB will be assessed by evaluating cells from systemic and mucosal tissues for the expression of co-stimulatory molecules and the profile of cytokines induced. 3) Determine if chimeric proteins consisting of cloned antigens of mutans streptococci are more effective than each antigen alone in inducing protective immune responses. Levels and isotype of antibodies to the cloned antigens in saliva and serum will be measured in animals immunized by the oral or IN route to determine if chimeric proteins of mutans streptococci antigens induce higher salivary IgA antibody responses and greater protection against infection by mutans streptococci than each cloned protein alone. The results will be relevant to establish the practicability of *Salmonella* vaccine delivery systems

and the usefulness of genetically derived chimeric proteins from virulence factors of a pathogen and adjuvants for the induction of protective immune responses against mucosal pathogens including those associated with the oral cavity.

Grant: 2R01DE009821-11
Program Director: MANGAN, DENNIS F.
Principal Investigator: KURAMITSU, HOWARD K PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: Oral spirochetes: molecular genetic analysis
Institution: STATE UNIVERSITY OF NEW YORK AT AMHERST, NY
BUFFALO
Project Period: 1992/02/20-2005/06/30

DESCRIPTION: This application will continue to examine the molecular basis for the virulence of spirochetes. Utilizing *Treponema denticola* mutants constructed in the previous grant period, the respective roles of motility, chemotaxis, and protease activity in the formation of biofilms will be investigated. In addition, a RNA differential display approach will be used to identify genes, which are involved in colonization of solid surfaces, preformed biofilms of *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, and host cell surfaces. These approaches will help to define the mechanisms, which oral spirochetes utilize in colonizing the subgingival margin. Furthermore, since penetration of tissue barriers by spirochetes appears to be an important virulence factor of these organisms, the molecular basis for this property will be examined in *T. denticola*. Using the HUVEC transcytosis system, genes of the spirochete, which are involved in tissue penetration, will be identified using genetic approaches. It will then be of interest to determine if the syphilitic organism *T. pallidum* utilizes a homologous set of genes to invade tissue. In addition, if the later organism utilizes species-specific invasion genes, the hypothesis that oral spirochetes, which are related to *T. pallidum*, may play a role in periodontitis will be examined. These approaches should increase understanding of the molecular basis for the pathogenicity of spirochetes.

Grant: 2R01DE010174-05A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: QUIVEY, ROBERT G PHD
MICROBIOLOGY:MICROBIO
OGY-UNSPEC
Title: LOW PH INDUCIBLE DNA REPAIR IN STREPTOCOCCUS MUTANS
Institution: UNIVERSITY OF ROCHESTER ROCHESTER, NY
Project Period: 1996/05/01-2005/11/30

DESCRIPTION: The ability to adapt to acidic conditions is a primary mechanism that *Streptococcus mutans* employs to survive the low pH environments that can occur in dental plaque. As part of that adaptive ability, the organism increases production of an enzyme, known as an exonuclease, which begins the repair of its DNA damaged during growth under acid conditions. Homologues of the enzyme, in other bacteria, are known to play a major role in the removal and repair of DNA damaged by oxidative agents, such as hydrogen peroxide. The investigators, and others, have shown that growth at low pH increases the ability of *S. mutans* to resist hydrogen peroxide. Thus, there is a link in *S. mutans* between resistance to acid conditions and the repair of damage caused by oxidative agents. Since many healthcare products now contain hydrogen peroxide, it is of interest to determine the mechanisms and abilities that *S. mutans* has available to resist its effects. Moreover, it appears that the organism has evolved mechanisms that are cross-protective, or over-lap, in their regulatory schemes. The objectives of the present application are to clarify how the organism becomes resistant to oxidative attack when it is growing in acidic conditions. The investigators will determine the regulatory mechanism and the biochemical abilities of the exonuclease to protect *S. mutans* from acidic and oxidative attack. The investigators goals will be accomplished by developing additional mutant strains of the bacteria; by developing site-specific mutations in the exonuclease; and by determining the mechanism by which *S. mutans* regulates exonuclease production at the level of transcriptional control. Specific Aims for the project are as follows: 1. Determination of how acidic conditions and oxidative damage act to induce, exonuclease synthesis and a determination of the identity of other participants, in addition to exonuclease, in the inducible repair system; 2. Definition of the kinds of damage that the exonuclease can recognize in DNA and the nature of the exonuclease structure that permits the enzyme its flexibility; 3. Elucidation of the regulatory mechanism for the *S. mutans* exonuclease, including a description of the genetic elements that participate in regulating synthesis of the enzyme and the protein(s) that function to regulate transcription of the exonuclease gene.

Grant: 2R01DE010362-10
Program Director: MANGAN, DENNIS F.
Principal Investigator: BURNE, ROBERT A
Title: Molecular Biology of Oral Alkali Production
Institution: UNIVERSITY OF FLORIDA ROCHESTER, NY
Project Period: 1992/08/01-2006/06/30

DESCRIPTION (provided by applicant): Alkali generation, in the form of ammonia, is a major impediment to the initiation and progression of dental caries. There is also indirect evidence to support that ammonia production impacts calculus deposition by promoting mineral precipitation, and it may also exacerbate periodontal diseases by impairing the function of normal host immune and repair processes. There are two major sources of ammonia in the mouth: urea and arginine, which are hydrolyzed by ureases and the arginine deiminase system (ADS) of oral bacteria, respectively. During the previous funding periods, substantial insight was gained about the molecular architecture, genetic regulation, the role of ureases of oral bacteria in physiologic homeostasis and the importance of alkali generation in caries inhibition. This proposal builds on our previous studies with the ureases of oral bacteria, focusing on two fundamental areas directly related to the molecular biology, physiology and role in oral diseases of ammonia production. The first continues the studies we have developed during the previous funding periods on the molecular biology of urea catabolism by oral microorganisms and the second goal is to thoroughly characterize the arginine deiminase systems (ADS) of two oral streptococci. To accomplish our goals, we have organized the project under two specific aims: Aim 1. Continued analysis of the genetics, physiology and role in oral ecology and disease of bacterial ureases focusing primarily i) on the pH- and carbohydrate-dependent expression of the urease of *Streptococcus salivarius*, but also ii) on the utility of recombinant, urease-producing bacteria in inhibition of dental caries and iii) on factors that may affect the ability of oral microorganisms to carry out ureolysis in the human oral cavity. Aim 2. Molecular analysis of the arginine deiminase system of *Streptococcus gordonii* and *Streptococcus rattus* focusing i) on the cis- and trans-acting factors governing induction by arginine, and repression by glucose or oxygen, ii) analysis of the role of the ADS in inhibition of the initiation and progression of dental caries using of ADS-deficient mutants of *S. gordonii* and *S. rattus*, or recombinant, arginolytic *S. mutans*, and iii) physiological analysis of arginine transport and analysis of the effects of fluoride on alkali-generation via the ADS. This research will provide insights into new ways to control caries and other oral infectious diseases by manipulating the capacity of oral microorganisms to produce ammonia and to modulate the pH of oral biofilms.

Grant: 2R01DE010510-06
Program Director: MANGAN, DENNIS F.
Principal Investigator: DUNCAN, MARGARET J PHD
Title: Functional genomics of *P. gingivalis*-host interactions
Institution: FORSYTH INSTITUTE BOSTON, MA
Project Period: 1996/08/01-2006/06/30

The broad, long-term goal of the research conducted under DE-10510 is to understand, at the molecular level, bacterial responses triggered by bacteria-host interaction; knowledge that is central to our comprehension of microbial pathogenesis and host defense. The first step in *Porphyromonas gingivalis* infection is colonization of the junctional epithelium lining the gingival sulcus. The objective of the proposal was to identify *P. gingivalis* genes and proteins that determined this initial interaction. A direct result of the study was the demonstration that gingipains were critical players in adherence of the bacterium to human epithelial cells. These important discoveries set the stage for new hypotheses that address the functional genomics of adhesion. Specific Aim 1 of this application tests the hypothesis that specific gingipain domains determine *P. gingivalis* adhesion to, and detachment from, epithelial cells. This will be tested with purified recombinant gingipain proteins and peptides in adhesion competition and blocking experiments. Specific Aim 2 addresses the hypothesis that a complex of proteins is involved in adhesion, and components of the complex will be identified. Specific Aims 3 and 4 introduce a new research area centered on the hypotheses that during infection a defined set of *P. gingivalis* genes are expressed to promote disease and survival of the organism in the face of host defenses; and the expression of non-essential genes is down-regulated to ensure the efficient use of cell resources. Thus, in Specific Aim 3, subtractive hybridization techniques will be used to examine gene expression after a defined stimulus, contact with epithelial cells. Specific Aim 4 continues this investigation with genome-wide analyses using state of the art *P. gingivalis* microarrays. Within the context of increased bacterial resistance to antibiotics, and the emergence of new human pathogens, an understanding of the molecular interactions between an infecting bacterium and its host is the accelerant to new drug discovery. Validation of our hypotheses regarding gingipains and epithelial cell adhesion may further stimulate searches for second-generation antibiotics directed to these proteinases. The discovery of new activities related to the pathogenic process will increase the repertoire of future targets, and drive the hunt for novel therapeutic interventions.

Grant: 2R01DE011664-05A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: STASHENKO, PHILIP P DMD DENTISTRY
Title: Immunity to Disseminating Dentoalveolar Infections
Institution: FORSYTH INSTITUTE BOSTON, MA
Project Period: 1996/02/01-2005/01/31

DESCRIPTION: (Verbatim from application) Dentoalveolar abscess causing bacteria evade ingestion and destruction by phagocytic leukocytes and other local host immune mechanisms. If not promptly treated, these infections may rapidly spread along fascial planes, through lymphatics and via the bloodstream, resulting in significant morbidity and even mortality. Individuals with immunodeficiencies, or undergoing chemotherapy or immunosuppression, are particularly vulnerable. During the first grant period we developed a novel mouse model in which T and B cell deficient (SCID) mice with mixed anaerobic pulpal infections develop dentoalveolar abscesses, disseminating infections and sepsis. B cell deficient mice were also susceptible but with reduced frequency, whereas T cell deficient mice were resistant. Dissemination was partially prevented by passive transfer of antibody. In this application we will elucidate the mechanisms that govern protection vs susceptibility to these infections. In Aim 1, the bacterial complexes that can cause disseminating infections in this model will be characterized. Aim 2 will determine the mechanism(s) of antibody - and phagocytic leukocyte-mediated protection against pathogens. In Aim 3, the mechanism(s) of increased susceptibility to disseminating infections and septic shock in SCID vs B cell deficient mice will be determined. The role of T cells and chemotactic cytokines in protection, or NK cells and shock-associated cytokines in increasing susceptibility will be determined in vivo. The goal of these studies is to fully define the immunological effector mechanisms that protect against disseminating dentoalveolar infections

Grant: 1R01DE013252-01A2
Program Director: MANGAN, DENNIS F.
Principal Investigator: RIVIERE, GEORGE R DDS
Title: SPIROCHETES INDICATE SUSCEPTIBILITY TO PERIODONTITIS
Institution: OREGON HEALTH & SCIENCE UNIVERSITY PORTLAND, OR
Project Period: 2001/08/01-2005/06/30

DESCRIPTION (Adapted from the Investigator's Abstract): The purpose of this research is to test the hypothesis that certain bacteria in plaque at sites of periodontal health are associated with the risk of periodontitis at infected sites. *Porphyromonas gingivalis*, *Prevotella intermedia*, *Treponema amylovorum*, *T. denticola*, *T. maltophilum*, *T. medium*, *T. pectinovorum*, *T. socranskii*, *T. vincentii*, and any new species of *Treponema* isolated from study sites that develop periodontitis will be evaluated as risk factors for disease. A quantifiable PCR assay will be used to further define risk by associating numbers of target bacteria with disease. Specific Aim 1: Correlate infection of health-associated plaque by each of seven oral *Treponema* species, *P. gingivalis*, *P. intermedia*, and new *Treponema* isolates with development of periodontitis. These studies will determine which disease-associated bacteria infect health-associated plaque before clinical detection of periodontitis. Specific Aim 2: Isolate *Treponema* from new sites of periodontitis at study sites. One of the designated target species not previously evaluated, or a yet-to be discovered *Treponema*, may be an important risk factor for the transition from gingival health to periodontitis. Specific Aim 3: Use a quantifiable PCR to estimate numbers of study bacteria in plaque. Risk for disease may be associated with critical numbers of specific bacteria in health-associated plaque before periodontitis is detected. The long-term goal of this research is to develop a means by which sites of periodontal health at risk for disease can be identified and treated before deterioration occurs.

Grant: 1R01DE013325-01A2
Program Director: MANGAN, DENNIS F.
Principal Investigator: DARVEAU, RICHARD P PHD
IMMUNOLOGY/IMMUNOPATHOLOGY
Title: LBP/CD14 interactions with bacterial components
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2001/04/15-2006/03/31

DESCRIPTION (Verbatim from Applicant's Abstract): Innate host inflammatory response mechanisms normally prevent microbial infection. In the clinically healthy periodontium, low level expression of select inflammatory mediators has been observed and is believed to provide inflammatory surveillance, protecting this tissue which is constantly exposed to bacteria. In periodontitis, the destruction of the tissue and bone surrounding the tooth root surface which is characteristic of this disease is believed to be due to high level expression of numerous innate host inflammatory mediators. Associated with the development of adult type periodontitis, the most common form of the disease, there is a characteristic shift in the dental plaque biofilm flora from mostly gram positive to mostly gram negative bacteria. The clinical correlation to disease associated with this shift is strong, however, it is not understood how these bacterial population changes influence the inflammatory response. The LBP / CD14 / Toll like receptor (TLR) system has been shown to facilitate innate host inflammatory responses to a wide variety of different bacteria. These key innate host defense proteins respond to both LTA from gram positive organisms and LPS from gram negative bacteria. Evidence suggests CD14 and TLR's may act together in regulating the intensity of inflammatory mediator production in response to different bacteria. CD14 interacts with a wide variety of different microbial ligands effectively concentrating them and "presenting" them to other innate host defense components such as TLRs. In contrast different TLRs are engaged with different microbial ligands resulting in activation of host cellular responses. However, the molecular mechanisms by which CD14 recognizes numerous different bacterial components and the contribution of specific structural features of LPS or LTA to CD14 or TLR interactions are not completely understood. Our overall hypothesis is: The innate host defense system recognizes bacteria in part by structural features present on lipopolysaccharide (LPS) and lipoteichoic acid (LTA) and modulates the inflammatory response accordingly. In this proposal, the structural features of LPS and LTA that influence CD14 and TLR binding and activation will be examined. We will determine the role of specific CD14 residues in LPS and LTA binding and transfer to TLR-2 and TLR-4, and determine the contribution of TLR-2 and TLR-4 to host cell activation with different structurally defined microbial ligands. These studies will provide further insight into how the innate host defense system recognizes and responds to different bacteria, a key component of both oral health and disease.

Grant: 1R01DE013565-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: FENNO, J CHRISTOPHER PHD MICROBIOLOGY &
MOLEC. GENETICS
Title: MOLECULAR ANALYSIS OF T. DENTICOLA-HOST INTERACTIONS
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2001/04/15-2005/01/31

DESCRIPTION: The predominance of spirochetes in subgingival plaque associated with severe periodontal lesions suggests an important role in periodontal pathogenesis. The goal of this research is to characterize interactions of *Treponema denticola* with subgingival tissues at the molecular level. By focusing on analysis of surface-expressed proteins that directly affect host cells, insights will be gained into mechanisms of periodontal cytopathology. The major outer membrane protein (Msp) of *T. denticola* binds to cells and ECM components, and has pore-forming cytotoxic activity. Msp is genetically conserved in many oral spirochetes, yet shows considerable inter-strain heterogeneity, suggesting that it is an important immunogen. The overall hypothesis is that Msp is a significant virulence determinant in periodontal disease, and is a key component of an outer membrane protein complex mediating interactions of the spirochete with subgingival tissue. Specific Aims of the proposed research, and the individual hypotheses to be tested are: 1) to characterize *T. denticola* proteins associated with Msp expression. Outer membrane components other than Msp are required for native Msp expression and assembly of the native outer membrane complex. Isogenic mutants and recombinant expression systems will be used to characterize these processes. 2) to identify immunodominant and functional domains of Msp. Antigenic heterogeneity of Msp is a factor in host antibody recognition of oral spirochetes. Archived serum samples will be screened for reactivity with specific Msps. Genes encoding novel Msps will be identified in patient plaque samples. 3) to characterize the role of Msp in cytopathic cellular responses to *T. denticola*. The ability of parent and msp mutant strains to bind host cells, ECM and serum components, and to activate pro-inflammatory cellular responses will be assayed. A putative Msp receptor identified on epithelial cell surfaces will be characterized. These studies, which involve both genetic and biochemical analyses, are intended to contribute significantly to the understanding of microbe-host interactions in the etiology of periodontal diseases, and to basic knowledge of the molecular biology of pathogenic spirochetes.

Grant: 1R01DE013657-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: ACTIS, LUIS A PHD BIOCHEMISTRY
Title: IRON UPTAKE IN ACTINOBACILLUS ACTINOMYCETEMCOMITANS
Institution: MIAMI UNIVERSITY OXFORD OXFORD, OH
Project Period: 2001/08/01-2005/06/30

DESCRIPTION (adapted from the Investigator's abstract): Localized juvenile periodontitis (LJP) is the disease that occurs when bacteria accumulate on the junctional epithelium in the oral cavity. It was found in the middle of the last decade that *Actinobacillus actinomycetemcomitans* is the major causative agent of LJP and, more recently, as one of the microorganisms responsible for adult periodontitis. Periodontitis is the most prevalent chronic inflammatory diseases in humans, and it is major cause of tooth loss. It is evident that this dental pathogen grows actively and persists during the infectious process, which depends upon the ability of this microorganism to obtain growth-essential nutrients such as iron. Iron withholding by vertebrate hosts is an efficient mechanism against bacterial infections, and, thus bacteria must express efficient transport systems to acquire this essential nutrient to multiply during infection. There are indications that *A. actinomycetemcomitans* does not secrete siderophore compounds and it may acquire iron through periplasmic-binding protein-dependent transport systems. However, the genes and bacterial products involved in these processes and their role in virulence remain to be characterized. Therefore, the long-term objective of this application is the characterization of the mechanisms that *A. actinomycetemcomitans* uses to acquire iron and their participation in the pathogenesis of juvenile and adult periodontitis. In this proposal, the Principal Investigator addresses these goals through several approaches, combining methods used in classical and molecular bacterial genetics with molecular biology techniques designed to examine differential gene expression. The first specific aim involves a detailed genetic and molecular characterization of two potential iron periplasmic-transport systems, while the second specific aim focuses on the analysis of the expression of these systems in bacterial cells cultured in bacteriological media and in tissue culture flasks containing monolayers of human oral epithelial cells. The third specific aim proposes to determine the role of these potential iron transport systems in iron acquisition and the virulence of *A. actinomycetemcomitans* by creating and testing isogenic mutants. These proposed studies address an important and largely unexplored aspect of the pathogenesis caused by *A. actinomycetemcomitans*. Furthermore, these studies will lead to a better understanding of the nature of the interactions between the host and this pathogen during colonization and invasion of oral tissues.

Grant: 1R01DE013725-01A2
Program Director: MANGAN, DENNIS F.
Principal Investigator: KAPILA, YVONNE L PHD
Title: Apoptosis Regulated by Fibronectin and Its Receptors
Institution: UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA
Project Period: 2001/08/15-2005/06/30

The goal of these studies is to understand the mechanism by which proteolytic fragments of fibronectin (FN) mediate apoptosis in periodontal ligament (PDL) cells. Although the etiology of periodontal disease has been attributed to bacterial pathogens, several components in the pathogenesis of this disease remain poorly understood. One such area is the role of matrix fragments generated by bacterial proteinases and by inflammatory host-derive enzymes in the progression of periodontal disease. Proteases expressed by several putative periodontal pathogens readily cleave FN into multiple fragments, which are found in vivo and in association with periodontally disease sites. One such fragment is a 40 kDa chymotryptic fragment which contains the heparin-binding domain and part of the alternatively spliced V region of FN and can be generated by the chymotrypsin-like enzyme produced by *Prevotella intermedia*. Both this 40 kDa fragment and a longer recombinant (V+H-) fragment that also has the heparin-binding domain and the alternatively spliced V region of FN induce apoptosis in PDL cells. In addition, fragments of FN alter cell motility and enhance proteinase expression in PDL cells. These findings lead to the hypothesis that proteolytic fragments of FN generated by bacterial and/or host inflammatory cell proteinases affect several PDL cell functions including, survival, thereby exacerbating the degradation of periodontal tissues and contributing to disease progression. The specific aims are to: (1) Characterize the matrix parameters by which the 40 kDa FN fragment induces apoptosis in PDL cells; (2) Identify the cell surface receptors for the 40 kDa FN fragment, and for the 40 kDa-containing recombinant FN fragments that are involved in regulating apoptosis in PDL cells; and (3) characterize the signaling pathways by which the 40 kDa FN fragment and other fragments containing the 40 kDa region regulate apoptosis of PDL cells. These findings will contribute to our understanding of the pathogenesis of periodontal disease and to our basic understanding of the regulation of apoptosis by the extracellular matrix.

Grant: 1R01DE013747-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: STASHENKO, PHILIP P DMD DENTISTRY
Title: INNATE IMMUNITY AND PERIODONTAL DISEASE IN MICE
Institution: FORSYTH INSTITUTE BOSTON, MA
Project Period: 2001/04/15-2005/01/31

DESCRIPTION: This is a revised application to study the role of elements of innate immunity in the pathogenesis of periodontal disease. Specifically, a novel model of P- and E-selectin deficient mice (P/E(-/-)) will be utilized to investigate the role of innate immunity, specifically, Toll-like receptors (TLR), in periodontal disease. Previous studies have shown that P/E(-/-) mice develop a progressive periodontitis that is initiated shortly after tooth eruption, and is characterized by an oral flora that is increased in mass and pathogenicity, gingival inflammation, increased expression of the bone resorptive cytokine IL-1, and extensive bone loss. Moreover, antibiotic treatment completely prevents bone loss. It is suggested that this model offers advantages over other systems, including the naturally occurring nature of the disease, the rapidity of periodontal destruction, the ability to control and manipulate the oral flora and the host immune response, and the availability of a vast array of reagents and genetically-engineered strains. The investigators will test the hypothesis that periodontal destruction can be ameliorated by modulating TLRs, their signaling pathways, and the cytokines that they induce. The proposed study is divided into four Specific Aims: 1) to identify the periodontal pathogens that are responsible for disease in P/E(-/-) mice; these studies will utilize 16S rRNA sequencing to characterize the oral flora in P/E(-/-), P/E(+/+) , and antibiotic treated mice; 2) to determine the immune mechanisms activated by pathogens in P/E(-/-) mice. Cell infiltrates, cytokines and Toll-like receptors (TLRs) will be characterized in vivo and in vitro; 3) to determine the function of TLRs in cytokine and co-stimulatory molecule expression in response to pathogens. Dominant negative constructs of TLR signal transducing molecules will be used to inhibit TLR responses and the effect on pathogen-induced cytokine responses determined. Also, the role of TLRs in skewing the immune response towards a Th1 and Th2 profile will be assessed; 4) to determine the roles of TLRs and cytokines in periodontal bone destruction. Knockout mice and modulation of IL-1, IL-6 and IL-10 will be used to establish the role of these factors in periodontal bone loss. The long-term goal of these studies is to determine the role of innate immunity in periodontitis and to apply this information to the development of immune modulators that ameliorate disease.

Grant: 1R01DE013824-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: MINTZ, KEITH P MS
Title: Molecular interactions: oral bacteria & matrix proteins
Institution: UNIVERSITY OF VERMONT & ST AGRIC BURLINGTON, VT
COLLEGE
Project Period: 2001/08/01-2004/06/30

Gram-negative bacteria are associated with periodontal diseases, which are a group of chronic inflammatory diseases of the gingiva and the supporting structures of the periodontium. *Actinobacillus actinomycetemcomitans* (Aa) is a Gram-negative, facultative coccobacillus that colonizes the human oral cavity and upper respiratory tract. This bacterium is closely associated with periodontitis in young individuals and with cases of adult periodontitis. This pathogen has been associated with other serious human infections such as endocarditis, soft tissue abscesses, and more recently cardiovascular disease. Although the periodontium is believed to be the source of these extraoral infections, little is known about the tropisms used by Aa to maintain itself within the oral cavity and to infiltrate and disseminate in tissues. Pathogens have evolved diverse strategies to be successful in colonization of the host tissue. A common theme amongst these pathogens is the ability to initiate infection by adhesion to specific host macromolecules under stringent or hostile conditions. These molecules include proteins that are secreted by host cells that form the extracellular matrix. This matrix is usually composed of collagen and specific noncollagenous proteins, e.g. fibronectin. The major protein found in the periodontium is collagen and we have demonstrated that Aa binds to both collagen and fibronectin. In this proposal, we plan to identify the genes coding for matrix binding proteins and determine the amino acid sequences of these proteins required for binding. It is our hypothesis that the synthesis of matrix binding proteins is involved in Aa colonization of the periodontal pocket and underlying tissues. In order to realize these goals, we propose to 1) isolate the genes coding for collagen and fibronectin binding proteins by constructing a transposon mutagenesis and a phage display library; 2) determine the gene sequences and generate isogenic mutants; 3) determine the regions(s) of the protein essential for binding activity; 4) determine the immunoreactivity of LJP patient sera to these proteins. This information can be used for the development of therapeutic agents of vaccines for periodontal disease.

Grant: 1R01DE013833-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: CONNELL, TERRY D PHD
Title: TYPE II Enterotoxins as Mucosal Immunomodulators
Institution: STATE UNIVERSITY OF NEW YORK AT AMHERST, NY
BUFFALO
Project Period: 2001/04/15-2005/03/31

DESCRIPTION: The objective of this application is to evaluate the mucosal adjuvant activities of the Escherichia coli Type II enterotoxins, LT-IIa and LT-IIb. Experiments in the laboratory of the applicant demonstrated that LT-IIa and LT-IIb induce different and distinctive patterns of enhanced immune responses, and that those patterns are profoundly different from those induced by cholera toxin (CT). For example, whereas CT used as an adjuvant induces predominantly a T helper 2-type response based on antibody isotype and cytokine patterns, Type II enterotoxins, particularly LT-IIb, induce both T helper 1 and T helper 2 responses. These data provide strong evidence that LT-IIa, LT-IIb, and CT induce their adjuvant activities using different cellular and molecular mechanisms. As such, the Type II toxins provide an elegant set of tools for investigating the mechanisms of mucosal adjuvant induction. Although related in structure, LT-IIa, LT-IIb and CT bind to different sets of cell surface receptors. It is hypothesized that the distinctive adjuvant activities of the toxins are governed by their receptor-binding specificities. To test this hypothesis, the adjuvant activities of the Type II toxins will be analyzed in a mucosal mouse model using AgI/II of the oral pathogen Streptococcus mutans as a model antigen. Both antibody and cellular responses will be assessed. These studies will be facilitated by a collection of receptor-binding mutants, hybrid molecules, and chimeric toxins that are available in this laboratory. Immunization studies will be combined with immunohistological investigations of lymphoid tissue to begin to investigate the cellular component of toxin-induced adjuvant activity. Confocal microscopy will be used to identify the immunocompetent cells in the nasal lymphoid tissue and the draining lymph nodes that initially interact with the toxins after intranasal inoculation. As a further means to correlate adjuvant induction with toxin/cell interactions, immunocompetent cells taken from nasal lymphoid tissue will be classified for expression of toxin-specific surface receptors using flow cytometry analysis. Finally, the potential of non-toxic chimeric Type II proteins as adjuvant/antigen delivery vehicles will be evaluated. At the conclusion of these studies, the laboratory will be well positioned to evaluate the therapeutic potential of the Type II toxins as mucosal adjuvants in the subsequent production of new vaccines that will protect against pathogens that infect the oral, gastric and urogenital mucosae.

Grant: 1R01DE013965-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: GOODMAN, STEVEN D PHD
Title: Regulation of gtf Gene Expression in S mutans
Institution: UNIVERSITY OF SOUTHERN CALIFORNIA LOS ANGELES, CA
Project Period: 2001/08/01-2006/06/30

Streptococcus mutans is a causative agent of dental caries. Its ability to inflict damage is strongly linked to the production of long chain glucose polymers (glucans) derived from dietary sucrose which allow the bacteria possesses a family of genes that express enzymes called glucosyltransferases (GTF). There are three GTFs in S. mutans. They share 50 percent sequence identity and are encoded by the homologous gtfB, gtfC and gtfD genes. The gtfB and gtfC gene are found in direct repeat with a mere 198 base pairs separation between coding sequences. Although mutations in either gtfB or gtfC produce a colonizing deficient phenotype, little is known about their regulation. Until recently, the only effector known to regulate these genes was sucrose, the substrate of the GTFs. Supplemented sucrose transiently induces a 3-fold increase in a gtfB transcriptional fusion. Now we have demonstrated that both gtfB and gtfC show coordinated growth phase- dependent expression. For both genes, expression peaks prior to exponential growth and falls over 100-fold by early stationary phase. In this proposal, we intend to further study this phenomenon including identifying critical cis and trans acting elements and through mutagenesis determine how these elements affect gtf gene expression. Since these gtf genes are involved in the colonization process, the genetic elements that are found here will need to be examined in terms of the bacteria's pathogenic mechanisms.

Grant: 1R01DE014029-01A1
Program Director: MANGAN, DENNIS F.
Principal Investigator: CHAFFIN, WELDA L
Title: Candida albicans oral biofilm
Institution: TEXAS TECH UNIVERSITY HEALTH SCIS LUBBOCK, TX
CENTER
Project Period: 2001/09/01-2005/08/31

DESCRIPTION (provided by applicant): *Candida albicans* is a commensal that colonizes skin and mucosal surfaces including the oral cavity. The organism is also an agent of opportunistic disease of these surfaces as well as internal disseminated disease. Oral candidiasis is associated with derangements of the oral flora related with the acquisition of microbes by neonates and anti-bacterial therapy, oral prostheses, and host factors such as diabetes mellitus and HIV infection. Oral manifestations include pseudomembranous candidiasis (thrush) and denture stomatitis. Oropharyngeal infection is virtually an inescapable consequence of AIDS (96 percent patients) and frequently reoccurs. Denture stomatitis may affect 50 percent of complete denture wearers. The organism forms biofilms on mucosa, teeth and oral devices such as dentures, generally in association with oral bacteria. Compared to planktonic cells, organisms in biofilms have characteristics such as reduced susceptibility to antifungal drugs and the presence of an extracellular matrix. This study will test the hypothesis that unique characteristics associated with *C. albicans* biofilms are the result of altered gene expression in general cellular metabolism as well as biofilm specific gene expression. A model of saliva-coated denture acrylic established in this laboratory will be used. About 230 alterations in general cellular metabolism have been identified in biofilm compared to planktonic cells by exploiting the high homology between *Saccharomyces cerevisiae* and *C. albicans* and the commercial availability of gene arrays for *S. cerevisiae*. In Aim 1 this approach will be applied to examine expression temporally during biofilm formation and to other conditions of biofilm development using *C. albicans* DNA chips. In Aim 2, expression in in vitro biodiverse models will also be examined to identify genes inherently associated with biofilms as differentiated from those influenced by the biofilm environment. Expression of selected genes from the inherent biofilm expression class will be determined in vivo in organisms recovered from human saliva. Aim 3 will examine the role of biofilm-regulated genes such as TUP1 and EFG1 using genetically modified strains.

Grant: 1R01DE014214-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: RUDNEY, JOEL D PHD
Title: Extracrevicular Invasion by Periodontal Pathogens
Institution: UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN
Project Period: 2001/08/01-2006/06/30

Gingival infection with periodontal pathogens often persists after treatment, and mucosa are thought to be a reservoir for recolonization. Invaded mucosal cells may provide a protected environment for these fastidious anaerobes. Preliminary studies used fluorescent in situ hybridization (FISH) with universal and specific rRNA probes and confocal microscopy (LSCM) to detect *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and unidentified bacteria inside buccal cells from 23 of 24 subjects. This suggests that intracellular mucosal bacteria exist in a multi-species community which may maintain itself by modulating or overcoming host cell defenses, with exfoliated cells providing a protected route for bacterial transmission. Those postulates will be tested by these specific aims: 1.) Determine the composition of the mucosal intracellular community by probing for additional periodontal pathogens and other mucosal species. 16S rRNA temperature gradient electrophoresis will be used to design probes for uncultured species. Multicolor FISH will determine whether more than one species can occupy a cell. 2.) Determine whether intracellular bacteria modulate or overcome cell defenses in vivo by using multicolor FISH/LSCM to compare invaded and uninvaded cells for expression of cytokine and antimicrobial peptide mRNA. 3.) Determine whether exfoliated invaded cells may be a vector for transmission by using a tissue culture model. Washed mucosal cells will be suspended in autologous or heterologous clarified saliva with or without antibiotics, and then incubated with green fluorescent protein (GFP)-labeled KB cells. Transmission will be evaluated by multicolor FISH/LSCM. 4.) Determine whether the intracellular mucosal community can establish and maintain itself in the absence of the gingival crevice by using FISH/LSCM to look for such communities in prenatate infants, and to verify whether they occur in edentulous adults. 5.) Determine whether mucosal invasion protects pathogens from elimination in periodontal patients who require aggressive treatment, by using FISH/LSCM and multiplex PCR to compare mucosal and gingival colonization before and after scaling and root planing, topical chlorhexidine, and systemic antibiotics.

Grant: 1R01DE014215-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: KATZ, JANNET
Title: Mechanisms of Immune Modulation and Peridontal Disease
Institution: UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL
BIRMINGHAM
Project Period: 2001/09/01-2006/06/30

Periodontal disease is the result of the host response to periodontal pathogens such as *Porphyromonas gingivalis*. *P. gingivalis* is a black-pigmented, Gram-negative pathogen, which expresses various virulence factors implicated in disease. The overall goal of this project is to define the cellular mechanism(s) involved in the induction of host responses protective against *P. gingivalis* infection. The studies proposed will concentrate on delineating the mechanisms by which adjuvants modulate host responses to *P. gingivalis* antigens. Emphasis will be placed on the role of B7 co-stimulatory molecules in host responses and in infection by *P. gingivalis*, and on the involvement of cytokines, specific target cells, and other cell surface receptors. Specifically, there are plans to: 1) Determine the immunogenicity of the recombinant catalytic domain of the lysine-specific protease Kgp (rKgp-CAT) and the HagA repeat domain (rHAgrep) of *P. gingivalis*, and the effect of the B subunit of cholera toxin (CTB) and monophosphoryl lipid A (MPL) in modulating responses to these antigens following intranasal immunization. The level, isotype and IgG subclass of antibodies induced to HAgrep or Kgp-CAT in serum and external secretion\$ will be measured by ELISA. The effects of CTB and MPL in enhancing/shifting responses will be further characterized by measuring antigen-specific proliferation and cytokine production. The effect of the responses, especially the nature of the antibodies, on protection will also be determined. 2) Determine the mechanisms by which the adjuvants CTB and MPL modulate the induction of the immune response. These studies will determine the involvement of the co-stimulatory B7 molecules in the immunoenhancing ability of the adjuvants and in *P. gingivalis* infection, the target cells affected by the adjuvants and the association between MPL, the Toll-like receptors and B7 co-stimulatory molecules. Groups of mice deficient in B7-1 (B7-1^{-/-}), B7-2 (B7-2^j or both (B7-1/B7-2^{-/-}) molecules and normal controls will be immunized with HAgrep or Kgp-CAT with and without CTB or MIPL. The levels of antibody and antibody-secreting cells will be assessed by ELISA and ELISPOT method. The effect of adjuvants on the expression of B7 molecules and of CD4OL will be analyzed by FACS. Differential regulation of cytokines induced and proliferative responses will also be assessed. The involvement of B7 co-stimulation in *P. gingivalis* infection will be assessed in B7 deficient mice. These studies will provide information on the mechanisms by which mucosal adjuvants modulate host immune responses, the target cells through which their adjuvanticity is exerted and the involvement of co-stimulatory signals provided by B7 molecules and of Toll-like receptors. An understanding of these processes is crucial for the development of means to interrupt/prevent periodontal disease pathogenesis by *Porphyromonas gingivalis*. Knowledge on the host effects induced by a potential periodontal vaccine will lead to the development of the therapeutic manipulation of effector function leading to the amelioration or prevention of periodontal disease and associated systemic diseases.

Grant: 1R01DE014328-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: CUTLER, CHRISTOPHER W PHD IMMUNOLOGY /
MICROBIOLOGY
Title: Oral Pathogens and Dendritic Cell Subsets
Institution: STATE UNIVERSITY NEW YORK STONY STONY BROOK, NY
BROOK
Project Period: 2001/02/01-2005/11/30

DESCRIPTION (Adapted from the Investigator's Abstract): The Principal Investigator has proposed a novel overall hypothesis and approach to understanding the pathophysiology of adult periodontitis (AP), one of the most common of diseases that afflict the US population. While mortality of the dentition is the most familiar outcome of AP, its links with other more severe diseases, including coronary artery disease, respiratory diseases and pre-term labor cannot be ignored. These investigators have called attention to the many intriguing parallels between AP and contact hypersensitivity (CHS). CHS is among the most common of dermatoses that afflicts mankind and one of the most intensively studied of in vivo immune responses. Both AP and CHS target the host integument (gingiva or skin) and appear to involve the activation and sensitization of similar subsets of antigen capture and presenting cells, the dendritic cells. Dendritic cells have been termed "Nature's adjuvant," being more efficient at antigen-presentation than macrophages or B cells and the only antigen-presenting cells (APCs) that can stimulate naive T cells to proliferate. This immunostimulatory capacity can also have detrimental effects for the host, as typified by contact hypersensitivity (CHS) responses. Both AP and CHS involve a predominantly destructive T cell response mediated by both regulatory and effector T cells. These investigators have shown that *Porphyromonas gingivalis* is a unique pathogen in this regard, able to infect, sensitize, and activate dendritic cells in vitro and likely, in situ. Many questions about the role of *P. gingivalis*-sensitized dendritic cells in AP, however, remain unanswered. The present proposal will definitively establish, using in situ, ex vivo and in vitro approaches, the role of dendritic cells in adult periodontitis, particularly that induced by *P. gingivalis*. Moreover, these studies will characterize the interactions of *P. gingivalis* with dendritic cells and will further our knowledge of the pathophysiology of AP as it relates to CHS. Future studies, outside the purview of this proposal, will involve understanding the T cell response to *P. gingivalis*-activated dendritic cells.

Grant: 1R21DE013990-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: WU, CHRISTINE D PHD MICROBIOLOGY
IMMUNOLOGY
Title: GOLDENSEAL(HYDRASTIS CANADENSIS)REMEDY FOR ORAL DISEASES
Institution: UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL
Project Period: 2001/04/15-2003/03/31

DESCRIPTION (APPLICANT'S ABSTRACT): Complementary and alternative medicine (CAM) has recently gained popularity with the American public. Research validating CAM has focused mainly on the treatment and prevention of systemic medical diseases while less attention has been paid to oral diseases. Oral diseases including dental caries and periodontal disease, are a major cause of loss of work and school days. Chemical and mechanical means have been used to control dental plaque bacteria, the etiologic agent of caries and periodontal disease. However, none of the available agents is ideal and frequently cause adverse effects. This justifies further search and development of alternative agents from natural sources that are safe and effective. The North American plant, *Hydrastis canadensis* L. (Ranunculaceae), known commercially as "Goldenseal," has been used for centuries as an antiseptic to treat skin disorders and as an antidiarrheal, antiseptic, astringent, hemostatic, and vasoconstrictor agent. Goldenseal is one of the major phytomedicines ("herbal remedies") sold in health food stores and pharmacies in the U.S. Several mouthrinses and toothpastes containing Goldenseal are available on the market. Although claims have been made by the manufactures regarding its ability to fight gum diseases and prevent caries, no scientific data is available to substantiate these claims. The goal of the proposed research is to evaluate the potential of Goldenseal as a remedy in prevention and treatment of oral diseases and to maintain oral health. It is hypothesized that antimicrobial compounds that are safe for humans can be identified from *H. canadensis*. These compounds may have potential as dental prophylactic/therapeutic agents and may also serve as lead compounds for the subsequent design and synthesis of new agents that are even more effective than the existing ones. The Specific Aims of this study are: SA1: To isolate and identify active antimicrobial compounds from *H. canadensis* by activity-guided fractionation and characterization; SA2: To determine antimicrobial activity of the purified compounds against cariogenic and periodontal pathogens; SA3: To investigate mixtures of purified antimicrobial compounds from *H. canadensis* for synergistic antimicrobial activities; SA4: To correlate bioactivity of various commercially available Goldenseal-containing oral hygiene products with levels of active alkaloids identified in SA2. The proposed research is innovative in that it represents collaboration between an oral microbiologist and a natural product chemist that will assure the speedy discovery of novel or known active compounds from Goldenseal and will provide scientific explanation as to the remedy's efficacy. It will also serve as a model system for the evaluation of existing herbal remedies for their oral health related claims. This application of CAM research will help to achieve better oral health and oral disease prevention, one of the to priority areas of focus specified b the U.S. Public Health Service in "Health People 2000."

Includes RPGs (Research Project Grants)
Excludes Clinical Trials

Grant: 1R21DE014472-01
Program Director: CANTO, MARIA TERESA
Principal Investigator: MOORE, PAUL A
Title: Microbiology/Immunology of Periodontal Disease in T1DM
Institution: UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PA
PITTSBURGH
Project Period: 2001/09/30-2003/08/31

DESCRIPTION: (provided by applicant): We propose to evaluate the periodontal disease, microbiology and immunology of a large adult Type 1 diabetic cohort and previously identified health control population. In 1992-1994, the University of Pittsburgh's Oral Health Science Institute (OHS) completed periodontal assessments of 406 subjects being followed by the ongoing University of Pittsburgh Epidemiology of Diabetes Complications Study (EDC). We propose to continue our collaboration by reexamining subjects in this cohort, who will now be 30 to 55 years old. This exploratory proposal will determine the prevalence, incidence and 10-year progression of periodontal disease of Type 1 diabetic and nondiabetic control subjects, and characterize the periodontal microflora and immune response within these populations. The overall goals of this proposal are to assess the impact of periodontal risk factors (diabetes, smoking, age of onset, elevated glucose, etc.) on disease progression microbiotas and immune responses. Additionally we plan to explore the interrelationships between periodontal infections and the incidence of coronary artery disease complications in this high-risk population. Because we have 10-year baseline periodontal disease data, we will be able to determine not only the prevalence, but also the incidence and site-specific progression of periodontal disease within this adult Type 1 diabetic cohort and an age-matched nondiabetic control group. In collaboration with the microbiology laboratory at Forsyth Dental Institute, we will be able to identify and quantify the gingival and periodontal microflora found within healthy and diseased sites of these populations. The impact of diabetic glycemic control (GhbA1, GhbA1-months, elevated fasting blood glucose) as well as salivary and GCF glucose concentrations will also be evaluated. The integration of this 10-year follow-up periodontal, microbiologic and immunologic assessment by the University of Pittsburgh's Oral Health Science Institute (OHSI) into this ongoing medical epidemiologic study (EDC) has several scientific and methodological benefits. The availability of a large Type 1 population having 10 years of baseline periodontal and medical data is a valuable asset. This inter-institutional collaboration has demonstrated reliable examination methodologies as well as the ability to share data management and statistical resources. An integrated multidisciplinary effort provides significant data collection capabilities essential for understanding the complex multifactorial etiology of periodontal disease in Type 1 diabetes.

Grant: 1R21DE014473-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: TENG, YEN-TUNG A PHD ORAL PATHOLOGY
Title: Immunity and Periodontal Breakdown in Diabetic NOD Mice
Institution: UNIVERSITY OF WESTERN ONTARIO LONDON, ON
Project Period: 2001/09/30-2003/08/31

DESCRIPTION: (provided by applicant): Type 1-diabetes is caused by autoimmune attack of the pancreatic b-cell islets mediated by diabetogenic T cells, resulting in insufficient insulin production and hyperglycemia. Like in type 2 diabetes, type-1 diabetics develop periodontal infections (i.e. periodontitis) more frequently than non-diabetics do and clinical and epidemiological studies support that type-1 diabetes is a risk factor for periodontitis. Further, periodontitis has recently been shown to be associated with an increased risk for certain systemic disorders and may interactively modulate metabolic outcomes of diabetes. Around \$10-15 billion dollars per year are spent on periodontal diagnosis and treatment including the replacement of affected teeth in North America. However, studies regarding the fundamental "immunological basis" associated with the increased risk for severe periodontal breakdown in type 1 diabetes are lacking and practically unknown. The Non Obese Diabetic (NOD) mouse shares many clinical features of human type-1 diabetes and is an excellent animal model for studying type-1 diabetes. The current proposal uses the NOD mice to investigate the contribution of autoimmunity to periodontal anti-bacterial immunity and the specific aims are to study I) the contribution of the osteoclastogenic cytokine, osteoprotegerin-ligand (OPG-L,) and Th1/Th2 cytokines produced by oral microorganism-specific CD4+ T cells for periodontal inflammation and destruction induced by a well-know periodontal pathogen *A. actinomycetemcomitans* (Aa) in diabetic, non-diabetic and pre-diabetic NOD mice; ii) the frequency of Aa-reactive periodontal CD4+ T cells in diabetic, non-diabetic and pre-diabetic NOD mice; ii) the frequency of Aa-reactive periodontal CD4+ cells in diabetic, non -diabetic and pre-diabetic NOD mice during periodontal inflammation and destruction and iii) whether there is a significant role for the humoral immune response during periodontal infection under autoimmune conditions in diabetic NOD mice. The new information generated will provide the biological relevance of OPG-L in mediating periodontal destruction in type-1 diabetes and ii) the relationship between OPG-L expression, TH1/TH2 cytokine profile and humoral immunity for periodontal breakdown in type-1 diabetes. Importantly, the proposed research will provide the first clarification whether there is immunological basis for severe periodontal breakdown in type-1 diabetes. Thus, this tern will facilitate our understanding of the links and studying the mechanisms of periodontal infection type-1 diabetes.

Grant: 1R21DE014476-01
Program Director: ADESANYA, MARGO R.
Principal Investigator: FOUAD, ASHRAF F DDS
Title: Endodontic infections in type 1 diabetic hosts
Institution: UNIVERSITY OF CONNECTICUT SCH OF FARMINGTON, CT
MED/DNT
Project Period: 2001/09/30-2003/08/31

DESCRIPTION: (provided by applicant): The causative microbial pathogens and the fundamental host responses in teeth with pulp necrosis and periapical (PA) lesions have not been adequately characterized. Much less is known about these host/pathogen interactions in patients with type 1 diabetes mellitus (DM). We hypothesize that patients with type 1 DM have more symptomatic and/or therapy-resistant PA lesions, which may or may not be related to the degree of their glycemic control. We also hypothesize that in root canals with necrotic pulp of these patients, more virulent and more numerous species of endodontopathic microorganisms are present. The aims of this research project are: (1) Determine the effects of type 1 DM on the development of symptoms in patients with pulp necrosis and apical periodontitis, and on the resolution of the periapical lesion after one year, and (2) Determine the effects of type 1 DM on the prevalence of pathogenic bacteria and *Candida albicans* before and after root canal preparation in these teeth, using sensitive molecular techniques. Endodontic patients who have type 1 DM or are non-diabetic, and who have at least one tooth with pulp necrosis and a periapical lesion, will be recruited for this study. Documentation of peri-operative symptoms will be done using visual analog scale measures for pain and swelling. Endodontic treatment will be completed in a standardized manner. Microbial samples from root canals of the teeth treated will be subjected to PCR amplification of the 16SrRNA gene of selected pathogenic bacteria or 18SrRNA gene *Candida*. Analysis will include universal eubacterial identification, followed by species-level identification of the selected pathogenic organisms using specific oligonucleotide PCR primers. Molecular sequencing will be performed on the PCR product generated with universal bacterial primers, in order to identify other root canal bacterial species present. Sampling and molecular identification will be repeated just before obturation of the root canals. Patients will have follow-up examinations one year post-operatively, with standardized periapical radiographs. Type 1 diabetic and non-diabetic patients will be compared as to peri-operative symptoms, treatment outcome, number of microbial species in the root canals preoperatively and following canal instrumentation, and the association of microbial species and their quantitative measures with symptoms and treatment outcome. Microbial and clinical factors will also be related to the degree of glycemic control of the diabetic patients.

Grant: 1R21DE014478-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: VAN DYKE, THOMAS E DDS DENTISTRY
Title: Periodontal Inflammation in Type 1 Diabetes
Institution: BOSTON UNIVERSITY MEDICAL CAMPUS BOSTON, MA
Project Period: 2001/09/30-2003/08/31

DESCRIPTION: (provided by applicant): Previous investigations of periodontal inflammation in diabetics have held to the paradigm that increased periodontal disease in diabetics is a result of depressed inflammatory cell function, including reduced neutrophil chemotaxis and reduced phagocytosis. New preliminary data from our laboratories, however, suggest that the diabetic neutrophil is actually primed or hyperactive. We have demonstrated that neutrophils obtained from diabetics exhibit increased superoxide and protein kinase C. Other data obtained in our laboratories have established that normal patients with periodontal disease mount a strong antibody response to *Porphyromonas gingivalis*, the primary etiological agent associated with periodontal disease, and in particular to the cysteine proteinases collectively referred to as gingipains. Furthermore, this antibody functions in the phagocytosis and killing of *p. gingivalis* by normal neutrophils. What is not known, however, is how the diabetic neutrophil responds specifically to *P. gingivalis* and to *P. gingivalis* components, including the gingipains. The objectives of this planning grant are to examine the neutrophil response to *P. gingivalis* and gingipains in Type 1 diabetic patients with periodontal disease. These studies are based on the hypothesis that increased periodontal disease in Type 1 diabetics is a sequel of increased neutrophil mediated tissue injury in response to *P. gingivalis* and to *P. gingivalis* components. The following specific aims are proposed: 1. To define the cellular and molecular basis of exaggerated neutrophil responses in Type 1 diabetics with periodontal disease as compared to diabetics free of periodontal disease, and normal, periodontally healthy, nondiabetic controls. a. We will evaluate neutrophil activation through measurements of superoxide production, production of cyclooxygenase-2 (COX-2), PGE2, and interleukin 1b (L-1b). These will be evaluated at both RNA and protein levels. b. We will define the specific signal transduction pathways leading to neutrophil activation ("on signals") and control of neutrophil activation ("off signals"). We will examine "on signals" including known proinflammatory receptors mediated by G-protein, phospholipase C and D, diglyceride, and protein kinase C pathways. The "off" signals will include lipoxin A4 pathways mediated by polyisoprenyl phosphate signaling. 2. To define the neutrophil mediated inflammatory response to *P. gingivalis* and the gingipains in the Type 1 diabetic patient. a. To evaluate phagocytosis and killing of *P. gingivalis* by neutrophils from diabetic patients with periodontal disease and appropriate normal controls. b. To examine the antibody response of diabetic patients with periodontal disease to *P. gingivalis* and the gingipains. c. To determine the contribution of the gingipains to the neutrophil response by evaluation of the opsonic activity of antibody to the arginine specific gingipains (RgpA and RgpB), and to the lysine specific gingipains (Kgp), for neutrophils from diabetics and normal controls.

Grant: 1R21DE014490-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: LALLA, EVANTHIA DDS
Title: Periodontal microbiota, serum antibody response & IDDM
Institution: COLUMBIA UNIVERSITY HEALTH SCIENCES NEW YORK, NY
Project Period: 2001/09/30-2003/08/31

DESCRIPTION: (provided by applicant): Diabetes mellitus is one of the most prevalent chronic diseases in the United States and worldwide. The persistent hyperglycemic state that characterizes type 1 diabetes leads to a number of significant complications that increase morbidity and mortality in affected individuals. Periodontal disease has been established as one of these complications. Recent research has begun to unravel some of the mechanisms involved in the development of diabetes-associated periodontal infections, but the basic underlying pathogenic mechanisms remain poorly understood. Interestingly, some supporting data also exist for an association between periodontal infections and atherosclerosis, and for an effect of periodontal therapy on the level of metabolic control in poorly controlled diabetic adults. However, additional evidence is needed, and the involved mechanisms need to be explored. In the context of advancing the diagnosis, prevention and treatment of periodontal disease in patients with type 1 diabetes, and taking advantage of an established relationship with the Naomi Berrie Diabetes Center on the Columbia Presbyterian Medical Center campus, we propose to examine a cohort of adult type 1 diabetic patients with a disease duration of 10 years. Non-diabetic individuals matched for gender, race, age and periodontal disease severity will be used as controls. We intend to investigate two key features of periodontal infections, namely the specific bacteria of the periodontal lesion and the systemic antibody responses to his bacterial challenge. We will examine the prevalence of a number of periodontal bacteria in subgingival plaque samples, including both putative pathogens and beneficial species, by means of the checkerboard DNA-DNA hybridization assay. We will further assess levels of serum antibodies to the above bacteria by the checkerboard immunoassay. Microbial and antibody profiles will be related to important diabetes-associated variables. In a subset of diabetes patients who exhibit the most severe periodontal conditions, we will investigate whether periodontal infection is associated with peripheral blood mononuclear cell activation. We will specifically assess levels of pro-inflammatory mediator of particular importance in atherogenesis, prior to and after mechanical periodontal therapy. These studies will markedly enhance our understanding of the oral microbiology and immunology in type 1 diabetes.

Grant: 1U01DE013905-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: GILL, STEVEN R PHD
Title: GENOME SEQUENCE OF STREPTOCOCCUS SOBRINUS
Institution: INSTITUTE FOR GENOMIC RESEARCH ROCKVILLE, MD
Project Period: 2001/09/01-2003/08/31

The goal of this project is to determine the genome sequence of *Streptococcus sobrinus* 6715, an oral bacterium associated with the formation of dental caries. *Streptococcus sobrinus* is one of the early colonizing oral bacteria that play a significant role in adherence to the tooth surface, and also in coaggregations with other types of bacteria to form plaque. Binding to the host derived protein layer that covers the tooth surface allows *S. sobrinus* and related streptococci to initiate colonization of the oral cavity. The highly aciduronic nature of *S. sobrinus* allows the organism to continue producing acid as the pH in its environment lowers. As acid production is important to the release of calcium phosphates from the tooth enamel, and the subsequent formation of caries, this unique ability of *S. sobrinus* to continue reducing environmental pH is significant to a complete understanding of periodontal disease. Recent advances in whole genome sequencing now make it possible to obtain the complete genome sequence of the 2.20 Mbp genome of *S. sobrinus* 6715. The complete sequence along with a complementary set of clones will provide a tremendous resource in being able to understand the biology of *S. sobrinus*. The proposed project will encompass: i) construction of random small and medium insert plasmid libraries and a large insert BAC library from *S. sobrinus* 6715; ii) sequencing of both ends of approximately 21,333 small and medium insert clones; iii) sequencing the ends of a set of BAC clones to provide a genome scaffold to order contigs, and reduce the effort needed for gap closure; and iv) assembly and annotation of the genome to identify structural features, and assign gene functional roles to open reading frames, based on database similarity searches. This whole genome sequencing project will complement the ongoing sequencing and analysis of the related streptococci *Streptococcus pneumoniae*, *S. pyogenes*, *S. agalactiae*, and *S. mutans*, and will allow for the identification of factors unique to these organisms. The project will also allow for the understanding of the pathogenic mechanisms used by *S. sobrinus* to successfully colonize the oral cavity. The data generated from this project will be deposited in public databases, including the TIGR World Wide Web Site. In addition, the libraries and overlapping BAC clone sets used to generate the complete genome sequence will be made available to the research community.

Grant: 1U01DE013914-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: GILL, STEVEN R PHD
Title: THE BACTEROIDES FORSYTHUS GENOME PROJECT
Institution: INSTITUTE FOR GENOMIC RESEARCH ROCKVILLE, MD
Project Period: 2001/09/01-2003/08/31

The gram negative anaerobe *Bacteroides forsythus* is consistently associated with chronic and severe adult periodontitis. Periodontal disease is routinely treated with surgery and tooth scaling. Determining the complete DNA sequence of the 2.2 Mbp genome of *B. forsythus* will identify all the open reading frames and other features associated with the genome. The DNA sequence and its annotation provide the information for functional genomic studies leading to the identification of targets for therapeutics and candidates for vaccine development. We will determine the complete genome sequence of the 2.2 Mbp genome of *B. forsythus* using a whole genome shotgun strategy. Small and large insert plasmid libraries will be prepared from *B. forsythus* randomly sheared genomic DNA. A sufficient number of random sequences (approximately 35,000 for 8-fold coverage) will be produced in TIGR's state of the art high throughput DNA sequencing facility. The sequence and physical gaps remaining following assembly and editing with TIGR's assembly and editing software (TIGR_Assembler ver 2.0, TIGR_Editor) are closed using a variety of sequencing and PCR strategies. Difficult repeat regions such as IS elements and rRNAs will be specifically PCR'd to insure the fidelity of the final genome structure and sequence. The DNA sequence data will be made available in compliance with the NIAID/NIDCR guidelines for large-scale genome sequencing projects. The complete genome sequence will be annotated using a variety of computer techniques. Open reading frames are identified with GLIMMER and searches of the predicted coding reading regions are done with BLASTP searched against a non-redundant bacterial protein database. Gene identification is enhanced by utilizing tools for multiple sequence alignment allowing us to build both orthologous and paralogous gene families. Additional structural features such as membrane spanning domains in proteins, untranslated RNAs, tRNAs, insertion sequences, and repeats will also be identified. The complete genome sequence and its analysis will be made available through the world wide web as part of the Comprehensive Microbial Resource (CMR) database developed at TIGR. We will also work closely with those investigators developing a specific Oral Pathogens database to make our data and analysis available.

Grant: 1U01DE013915-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: GILL, STEVEN R PHD
Title: GENOME ANALYSIS OF STREPTOCOCCUS GORDONII
Institution: INSTITUTE FOR GENOMIC RESEARCH ROCKVILLE, MD
Project Period: 2001/09/01-2003/08/31

DESCRIPTION (Investigator's Abstract): The goal of this project is to determine the complete genome sequence of *Streptococcus gordonii*, a pioneering colonizer of dental plaque and significant causative bacterium for infective endocarditis. The formation of dental plaque is regulated by complex interactions between the dental surfaces of the host and the surface structures of *S.gordonii* and other oral bacteria. The basis of the interacting surface structures is highly specific adherence mechanisms controlled by adhesin-receptor partnerships and extracellular glucans. Infective endocarditis often occurs as a result of oral trauma and is likely dependent upon the production of proteases and additional virulence factors. Genetic recombination and transfer of adhesins, antibiotic resistance determinants and possible virulent factors between the oral streptococci may be responsible for evolution of adhesins/receptor pairs and development of antibiotic resistance in *S.gordonii*. Finally, *S.gordonii* has been shown to be an effective antigen-delivery vehicle for vaccine development. Through the identification of genome structure, novel adhesions/receptors and regulatory elements, this project will accelerate experimental work directed towards understanding the dynamics of *S. gordonii* in dental plaque formation and its role in infective endocarditis. The approach to sequencing *Streptococcus gordonii* strain Challis (NCTC7868) will be a modified whole genome random sequencing strategy successfully used at TIGR to completely sequence 14 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. gordonii* strain Challis, 2) sequencing both ends of approximately 23,500 small and medium insert clones, 3) sequencing the ends of a set of minimally overlapping BAC 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 upon database similarity searches. The data developed from this study will be deposited in several databases, including the TIGR web site. In addition, all clone sets will be made available to the research community.

Grant: 1U01DE013927-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: HEIDELBERG, JOHN F PHD
Title: WHOLE GENOME SEQUENCING OF PREVOTELLA INTERMEDIA
Institution: INSTITUTE FOR GENOMIC RESEARCH ROCKVILLE, MD
Project Period: 2001/09/01-2003/08/31

Prevotella intermedia has also been associated with periodontal breakdown in Type I diabetics and with endodontic infections including those of the root canal, apical periodontitis, and periapical pathosis. Additionally, this bacterium has been implicated in extra-oral infections that include acute tonsillitis, bacterial tracheitis, and uvuhtis in children and more recently in the development of NOMA (cancrun oris), a debilitating, infectious disease that destroys the orofacial and neighboring structures. Many strains of P. intermedia possess hemagglutinating activity and some exhibit hemolytic activity. Furthermore, proliferation profiles suggest P. intermedia may possess "superantigenic" activity similar to staphylococcal enterotoxin A. Despite the association of P. intermedia with periodontal and other diseases and its ability to induce Tcell responses, only a limited knowledge of virulence features is available at the cellular and molecular levels. Evidence suggests that P. intermedia is one of the more drug resistant periodontal pathogens, which presents a formidable problem in determining effective treatments for the P. intermedia associated diseases. As a result, there is a critical need to develop genetic approaches to increase our understanding of the cellular/molecular biology and gene content, which will enable investigators to develop novel therapeutic approaches. A determination of the complete genome sequence represents the most effective approach towards realizing these goals. We propose to sequence the 2.8 Mb. Prevotella intermedia clinical isolate strain 17, a well-documented virulent strain. The complete genome sequence of P. intermedia will be determined using the whole genome random shotgun approach currently used at TIGR.

Grant: 1U01DE013930-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: TETTELIN, HERVE S PHD
Title: WHOLE GENOME SHOTGUN SEQUENCING OF STREPTOCOCCUS MITIS
Institution: INSTITUTE FOR GENOMIC RESEARCH ROCKVILLE, MD
Project Period: 2001/09/01-2003/03/31

DESCRIPTION (Adapted from the Investigator's Abstract): The goal is to determine the complete genome sequence of the type strain of *Streptococcus mitis*, the predominant pioneer colonizer of tooth surfaces, and thus an initiator of dental plaque formation relevant to caries and periodontal disease. These pioneer streptococci facilitate or inhibit the establishment of late colonizers and oral pathogens. The predominance of IgA1 protease producing *S. mitis* variants in the pharynx is associated with allergic disease. *S. mitis* is a causative agent of endocarditis. It is an emerging source of septicemia in cancer patients, and the first vancomycin-resistant *S. mitis* isolate was reported in one such case. Phylogenetically, *S. mitis* and the overt pathogen *S. pneumoniae*, the causative agent of otitis, pneumoniae, septicemia and meningitis, are nearest neighbors. There is clear demonstration that the *S. mitis* type strain constitutes a reservoir of genetic determinants that contribute to penicillin resistance in pathogenic *S. pneumoniae*. The fear is that resistance to vancomycin will be transferred in a similar manner. The approach is a whole genome random sequencing strategy used successfully at TIGR to sequence twelve prokaryotic genomes. The yearlong project will consist of four phases: 1) Construction of small, medium and large insert size genomic libraries from *S. mitis* type strain, NCTC 12261. 2) Sequencing both ends of a sufficient number of small insert clones to provide 8-fold sequence coverage of the genome. Medium and large insert clones will be sequenced to 6-fold and 10-fold clone coverage, respectively. This will provide a sequence scaffold that will aid in verifying genome architecture. Further, 10-fold clone coverage from the large (50 kb) insert library will ensure a predicted sequence hit every 5,000 bp of the 2.2 Mb *S. mitis* genome. 3) Assembling the genome sequence from the collection of sequence reads, and closing remaining gaps. 4) Annotation of the genome sequence to identify all open reading frames, assignment of gene names and functional roles based on database similarity searches. Accomplishing this goal will provide a unique opportunity to compare the repertoire of genes in the commensal *S. mitis* to that of other streptococci, such as the highly virulent, type-4 *S. pneumoniae* strain, nearing completion at TIGR, allowing the identification of virulence-associated genes.

Grant: 1U01DE013971-01
Program Director: MANGAN, DENNIS F.
Principal Investigator: READ, TIMOTHY D PHD
Title: THE WHOLE GENOME SEQUENCE OF ACTINOMYCES NAESLUNDII
Institution: INSTITUTE FOR GENOMIC RESEARCH ROCKVILLE, MD
Project Period: 2001/09/01-2003/08/31

DESCRIPTION (Adapted from the Investigator's Abstract): The goal of this two year project is to determine the sequence of the chromosome of the human oral bacterium *Actinomyces naeslundii*. *A. naeslundii* is the most prominent actinomyces sp. found in humans where its niche is almost exclusively the oral mucosa and root surfaces. *A. naeslundii* colonizes humans in the first few days postpartum to become an important component of the oral biofilm. The bacterium is a significant portion of the flora of healthy adults but it can also act as part of the matrix that is necessary for infection by specialized oral pathogens. The *A. naeslundii* strain to be sequenced is an isolate called MG1, which is the prominent *A. naeslundii* genospecies found in humans, and is also a strain that can be genetically manipulated. This strain's genome is estimated to be approximately 6.7 Mb. Small, medium and large insert libraries will be end sequenced to provide approximately 8X genome coverage. After alignment of about 100,000 sequencing reads using the TIGR assembler program, gaps will be filled in by directed PCR of genomic DNA. With generation of these clones and determination of the complete genome sequence, similarities in genes among other bacteria or in gene databases will be established using appropriate computer-assisted approaches, providing a working knowledge of absent or supplemental genetic components. These data will lead to the identification of novel genes of *A. naeslundii* potentially involved in attachment and colonization to human tissue, genes important in the pathogenesis of infections caused by the bacterium and determinants for surface proteins that are candidate targets for vaccines and other therapeutic interventions. In particular, this project will greatly aid in understanding the vital role *A. naeslundii* plays in the early colonization of the human oral cavity and in biofilm formation through interactions with other oral bacteria.

Grant: 2R01DK030292-20
Program Director: MAY, MICHAEL K.
Principal Investigator: GORDON, JEFFREY I MD OTHER AREAS
Title: Regulation of gene expression in the small intestine
Institution: WASHINGTON UNIVERSITY ST LOUIS, MO
Project Period: 1982/01/01-2006/03/31

DESCRIPTION (Applicant's Abstract): This proposal seeks continued support for the molecular analysis of the role of the intestine's nonpathogenic bacteria in gut development and function. The applicant has colonized adult germ-free mice with *Bacteroides thetaiotaomicron*, a genetically manipulatable, prominent member of the normal mouse and human ileal microflora. The investigator has already used high density oligonucleotide-based microarrays, laser capture microdissection (LCM) and real-time quantitative RT-PCR to show that 10 days after colonization, *B. thetaiotaomicron* affects expression of genes involved in a number of critical intestinal functions (e.g. nutrient processing and absorption, fortification of the mucosal barrier, xenobiotic metabolism, angiogenesis, plus genes associated with the enteric nervous system). Moreover, changes normally associated with the suckling-weaning transition are elicited in adult germ-free mice by *B. thetaiotaomicron*, suggesting that indigenous bacteria may play an instructive role in postnatal intestinal development. The PI wishes to pursue two specific aims. Aim 1 seeks to obtain a comprehensive molecular view of the impact of *B. thetaiotaomicron* on intestinal function and development. They will use a combination of germ-free mice, microarrays, LCM, real-time RT-PCR, in situ hybridization and immunohistochemical methods to examine the effect of *B. thetaiotaomicron* on intestinal gene expression as a function of time after initial colonization, position along the length of the intestine, and the presence or absence of mucosal T- and B-cells. The specificity of host responses will be explored by colonizing germ-free mice with another prominent member of the ileal microflora, *Bifidobacterium infantis*, and with the 'complete' ileal microflora of conventionally raised mice. The results will be used to develop, and genetically test specific hypotheses about contribution of selected host responses to normal intestinal development and function. Aim 2 proposes to identify microbial factors that regulate key host functions. They are in the process of sequencing the entire 4.8 Mb *B. thetaiotaomicron* genome. The investigators will identify ORFs using a variety of computational methods. Bacterial genes that are preferentially expressed in vivo will then be identified using promoter traps, as well as direct measurements of the levels of microbial mRNAs with custom microarrays. The results will then be used to design and conduct genetic tests of hypotheses about the role of selected *B. thetaiotaomicron* genes on intestinal responses to colonization. These studies should help determine whether microbes and their products can be used as therapeutic agents to improve nutrition, to promote proper gut development and physiologic functioning, and to prevent or treat various intestinal diseases.

Grant: 2R01DK039693-12A1
Program Director: MCKEON, CATHERINE
Principal Investigator: PRINCE, ALICE S
Title: CF EPITHELIAL CELLS: INTERACTIONS WITH PSEUDOMONAS
Institution: COLUMBIA UNIVERSITY HEALTH SCIENCES NEW YORK, NY
Project Period: 1991/07/01-2005/11/30

DESCRIPTION (Applicant's Abstract): The respiratory epithelium provides a major defense mechanism against the many pathogens that are inadvertently inhaled, responding with the expression of chemokines such as IL-8 and mucin. In cystic fibrosis, there is excessive inflammatory response to these organisms, particularly *P. aeruginosa*, which initiates the airway inflammation characteristic of this disease. In the experiments described, the ligands and receptors involved in this host-pathogen interaction will be defined in detail. Caveolae, spatially organized clusters of membrane glycolipids, scaffolding proteins, and signaling kinases appear to be important in mediating bacterial attachment and directing the epithelial IL-8 response. The components of caveolae that present the GalNAcGal bacterial receptor will be established, and how they may differ in cells with CFTR mutations will be explored. As several different *P. aeruginosa* ligands interact with epithelial components, additional receptors will be sought, including those which may activate other epithelial signaling cascades. Many of the known *P. aeruginosa* ligands are glycosylated, such as pilin, flagellin, and lipopolysaccharide (LPS). Exactly how glycosylation affects the adhesin function of these gene products or their ability to stimulate the host immune response is not well established. By using genetically defined mutants of *P. aeruginosa*, and comparing glycosylated and non-glycosylated ligands, especially flagella, we will determine how glycosylation affects bacterial-epithelial interactions, and if these carbohydrate structures may serve as targets for the development of therapeutic strategies. LPS is an important glycosylated virulence factor that activates both immune and epithelial cells. The role of specific carbohydrate components of *P. aeruginosa* LPS in pathogenesis will be examined and how LPS carbohydrate side chains alter host pathogen interactions in normal and CF cells will be explored. Other bacterial virulence factors, such as phospholipases, are important in activating epithelial responses. *P. aeruginosa* express several PLC'S which can interact with membrane components and activate cytokine signaling. Using genetically defined mutants, the contribution of PLC to epithelial damage in normal and CF cells will be examined.

Grant: 2R01DK048106-08A1
Program Director: HAMILTON, FRANK A.
Principal Investigator: LENCER, WAYNE I MD
Title: Intestinal Disease: Enterocyte/Toxin Interaction
Institution: CHILDREN'S HOSPITAL (BOSTON) BOSTON, MA
Project Period: 1993/09/01-2006/05/31

DESCRIPTION (Applicant's Abstract): Cholera toxin (CT) produced by *Vibrio cholerae* is the virulence factor responsible for the massive secretory diarrhea seen in Asiatic cholera. To induce disease, CT must bind ganglioside GM1 on the host cell apical membrane, enter the cell by endocytosis, and then somehow cross the cell to activate adenylyl cyclase on the cytoplasmic surface of the basolateral membrane. The investigator published data show that CT may move retrograde through Golgi and ER before arrival at the basolateral membrane, and that sorting into this pathway may depend on the lipid-based membrane anchor provided by the toxin's receptor GM1. GM1 concentrates CT in detergent-insoluble glycolipid-rich apical membrane microdomains (DIGs or "lipid rafts"). The applicant hypothesizes that GM1 endows CT with a lipid-based sorting motif that specifies association with DIGs and trafficking into the apical endosome, Golgi cisternae, ER, or transcytotic pathway. It is also not known how the toxin's enzymatic A-subunit enters the cytosol of host cells. Since CT must enter the ER for bioactivity, the investigator hypothesizes that C2 opportunistically utilizes the ER associated degradation system (ERAD) to unfold and cross the membrane via a protein translocase, possibly sec61p. To test these ideas, the applicant will define whether the ceramide domain of GM1 specifies the selective association with DIGs and toxin action in polarized T84 cells. The PI will use toxin variants deficient in clustering GM1 to test if cross-linking individual gangliosides is a prerequisite for association with DIGs or toxin function. The PI will examine raft dependence on membrane cholesterol by using beta-methyl-cyclodextrin, heterogeneity in GM1 content by using a CT variant attenuated in binding GM1, and functional association with the cortical cytoskeleton by membrane fractionation, disruption of actin filaments, and depletion of cholesterol. To test whether GM1 specifies toxin sorting into Golgi and ER, as opposed to the endosome-lysosomal or direct transcytotic pathway, the intracellular itinerary of CT (that binds GM1) and the closely related *E. coli* toxin LTIIb (that binds ganglioside GD1a) will be systematically compared. Toxin entry into the Golgi or ER, will be defined by microscopy and by exploiting the trans-Golgi specific transfer of sulfate and the ER specific transfer of N-linked oligosaccharides to label CT in these compartments. The mechanism of toxin-unfolding and dislocation from the ER to cytosol will be examined in vitro using purified ER luminal and membrane proteins, and in intact T84 cells through the use of selected toxin variants lacking sites for proteolytic nicking, ubiquitination and the cys187-199 disulfide bond in the toxin's enzymatic A-subunit.

Grant: 2R01DK050694-05A1
Program Director: HAMILTON, FRANK A.
Principal Investigator: HECHT, GAIL A MD
MEDICINE/GASTROENTEROLOGY
Title: EFFECTS OF E. COLI ON INTESTINAL FUNCTION
Institution: UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL
Project Period: 1997/06/01-2006/06/30

Enteropathogenic *Escherichia coli* (EPEC) is a major cause of diarrhea worldwide and is associated with high rates of morbidity and mortality. The mechanisms underlying EPEC pathogenesis are not understood. EPEC has direct effects on host intestinal epithelial functions including tight junction (TJ) permeability which is believed to contribute to diarrhea. The objective of this proposal is to elucidate the cellular and molecular basis for the EPEC-induced alterations in host intestinal epithelial tight junction barrier function. In part, the changes in intestinal TJ permeability are related to contraction of the perijunctional cytoskeletal ring. EPEC also alters TJ-associated proteins, including occludin, ZO-1, and claudin-1. Several TJ proteins directly interact with the cytoskeleton thus reconciling the observation that EPEC may exploit both mechanisms by which the TJ barrier is regulated. Experiments for Specific Aim 1 will characterize the effects of EPEC on individual TJ proteins and their interactions with each other. The focus of Specific Aim 2 will be to investigate the relationship between the two mechanisms by which EPEC perturbs the TJ barrier i.e., cytoskeletal contraction and alterations in TJ proteins. The proteins that transmit signals initiated by EPEC attachment to the host cell are not defined. Ezrin, a membrane-cytoskeleton linker molecule capable of mediating signal transduction events, may be involved in the cross-talk between microbe and host. Specific Aim 3 will define the role of the membrane-cytoskeleton linker protein ezrin in EPEC-induced alterations in tight junctions. EPEC virulence genes are likely involved in the perturbation of TJ barrier function since non-pathogenic *E. coli* do not elicit the same effect. The pathogenicity island of EPEC, called the locus of enterocyte effacement, has been sequenced and cloned and contains genes encoding type III secretory machinery, through which bacteria directly deliver proteins into host cells. Studies outlined in Specific Aim 4 will determine the specific EPEC proteins involved in the alteration of host intestinal epithelial TJ proteins and identify the signaling pathways responsible. These Specific Aims will address the overall hypothesis of this proposal which is that EPEC disrupts the tight junction barrier by stimulating contraction of the perijunctional cytoskeletal ring and by altering tight junction-associated proteins via signaling pathways transduced by the membrane-cytoskeletal linker protein ezrin. We further hypothesize that specific EPEC attachment factors and/or injection of proteins into host intestinal epithelial cells by type III secretion are responsible for this physiological alteration.

Grant: 2R01DK051131-06
Program Director: HAFT, CAROL RENFREW
Principal Investigator: KABACK, H RONALD MD BIOLOGY
NEC:BIOCHEMISTRY
Title: New Approaches to Membrane Protein Structure
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 1996/06/01-2005/04/30

A highly significant percentage of the genomes sequenced thus far are thought to encode polytopic transmembrane proteins which catalyze a multitude of essential cellular functions, energy and signal transduction in particular. Many are important with regard to human disease (e.g. cystic fibrosis, drug resistance), and many widely prescribed drugs (eg. Prozac and Prilosec) are targeted to membrane transport proteins. Although progress over the last 20 years has led to the characterization, purification and modification of this class of proteins, only a few have been studied at a level useful for understanding mechanism. Furthermore, many membrane proteins require conformational flexibility in order to function, making it imperative to obtain dynamic structural information. The objectives of this application are to continue to utilize the lactose permease of *Escherichia coli* as a paradigm for structure/function studies on transmembrane proteins. Only 6 amino acid residues are irreplaceable with respect to mechanism, and application of novel site-directed biochemical and biophysical approaches has yielded a helix packing model to a resolution approximating 4 Angstrom units. Further efforts will be made to refine and extend the structure using these methods. In addition, newly developed approaches using site-directed fluorescence resonance energy transfer and solid-state ^{19}F -NMR will be introduced. Ligand-induced conformational changes in certain helices can also be demonstrated, and these studies will be extended to the remainder of the molecule in order to delineate overall structural changes that result from ligand binding. The substrate binding site is located at the interface between helices IV and V, and specificity is directed towards the galactosyl moiety of the substrate. A spin-labeled galactoside that binds to the permease with high affinity has been synthesized and will be used to further define the substrate binding site. Ligands that bind but are not translocated are also being synthesized in order to study binding from the inner and outer surface of the membrane in the absence of translocation. Site-specific alkylation combined with mass spectrometry will be used to determine changes in the protonation of His322 (helix X) upon ligand binding.

Grant: 2R01DK052081-06
Program Director: HAMILTON, FRANK A.
Principal Investigator: TARR, PHILLIP I MD
Title: Pathophysiology of Childhood Hemolytic Uremic Syndrome
Institution: CHILDREN'S HOSPITAL AND REG MEDICAL SEATTLE, WA
CTR
Project Period: 1996/09/30-2006/08/31

DESCRIPTION (provided by applicant): Escherichia coli O157:H7-associated hemolytic uremic syndrome (HUS) remains a challenging medical problem. Extensive thrombogenesis, with accompanying fibrinolysis inhibition, precede the earliest indications of renal injury. Trends in thrombogenesis moreover predict the severity of ensuing HUS. Presumably, a massive endothelial injury before renal injury leads to HUS. This injury is amenable to study, and, possibly, to the attenuation of its effects. In this grant, the thrombogenic process in infected children will be studied intensively. Specifically, we will test the hypotheses that thrombogenesis clearly precedes renal injury, and that the rate of fibrin formation on initial assessment of E. coli O157:H7 infections is associated with outcome (Aim 1). To further assess the coagulation lesion, we will also test the hypotheses that net interval accumulation of fibrin between the first and second day of observation predicts outcome of this infection, and that time-dependent changes in prothrombotic kinetics underlie the pathophysiologic cascade leading to the development of HUS (Aim 2). We will also use our unique population to test the hypotheses that (a) the degree of activation of the complement system predicts outcome in children with E. coli O157:H7 infection, and (b) one or more factor H gene polymorphisms is associated with this outcome (Aim 3). Finally, because the endothelial cell is likely to be critical in the evolution of HUS, we will test the hypothesis that differences between the concentration of circulating endothelial cells in infected children predict outcome. We shall also test the subsidiary hypothesis that these cells express proteins plausibly related to their in situ injury or activation (Aim 4). A unique network has been assembled to identify children at risk of developing HUS an average of three days before HUS develops. This is an inadequately studied, but absolutely appropriate, model for toxin-related HUS. This network will be amalgamated with investigative expertise in the fields of coagulation assessment, complement pathophysiology and genetics, and endothelial cell biology. The project seeks a more complete understanding of the cascade leading to HUS, and, with this knowledge, the successful interdiction of this process.

Grant: 2R01DK053689-04
Program Director: HAMILTON, FRANK A.
Principal Investigator: PARSONNET, JULIE
Title: CLINICAL EXPERIMENT OF HELICOBACTER PYLORI TRANSMISSION
Institution: STANFORD UNIVERSITY STANFORD, CA
Project Period: 1997/09/30-2006/02/28

DESCRIPTION (Adapted from the Applicant's Abstract): Humans are the only known reservoir of *H. pylori* infection. How the organism is transmitted from one person to another, however, remains unknown. Data collected during the investigators initial funding period indicate that viable *H. pylori* are shed by infected hosts in vomitus and diarrheal stools under conditions that simulate gastroenteritis. In addition, *H. pylori* infection has been linked to increased risk for diarrheal disease, specifically, symptomatic cholera and infantile diarrhea. The investigators postulate that *H. pylori* decreases gastric acidity, allowing gastroenteritis pathogens to circumvent the first barrier to entry into the intestine. The gastroenteritis pathogens then cause diarrhea and vomiting, fostering excretion of *H. pylori* and completion of the transmission cycle. With this submission, they propose: 1) to determine whether *H. pylori* infection, by decreasing gastric acidity, is permissive of gastrointestinal infection with acid sensitive organisms. and 2) to determine whether gastrointestinal infection which leads to diarrhea and vomiting increases shedding of *H. pylori*. These aims will be accomplished in a three-pronged fashion: First, they will identify suitable acid-resistant and acid-sensitive strains of non-pathogenic *E. coli* for human inoculation, and determine the conditions for recovering these organisms from stools. Next, they will administer the acid-resistant/acid-sensitive pair to human subjects and determine the effects of *H. pylori* infection and gastric acidity on bacterial survival. Last, they will inoculate *H. pylori* infected and uninfected human subjects with low doses of an acid-sensitive, enteropathogenic *E. coli* (EPEC) and determine both how *H. pylori* affects EPEC infectivity and how EPEC affects *H. pylori* shedding. *H. pylori* infection causes gastric cancer-the second leading cause of cancer death worldwide-and peptic ulcer disease. Diarrheal disease remains a leading killer of children in developing countries, causing 20 percent of infant mortality worldwide. In these same countries where diarrheal disease runs rampant, *H. pylori* infects up to 80 percent of the population. If a causal link between these diseases can be established, then treatment or prevention of *H. pylori* would attain a significantly higher public health priority than it currently occupies.

Grant: 1R01DK058529-01
Program Director: MAY, MICHAEL K.
Principal Investigator: GORDON, JEFFREY I MD OTHER AREAS
Title: GNOTOBIOTIC TRANSGENIC MODELS OF THE GASTRIC ECOSYSTEM
Institution: WASHINGTON UNIVERSITY ST. LOUIS, MO
Project Period: 2001/02/01-2005/12/31

DESCRIPTION (Adapted from the Applicant's Abstract): This proposal examines 2 questions. (1) What are the molecular features of gastric epithelial lineage progenitors and how are they affected by parietal cell loss? (2) What role does attachment of *Helicobacter pylori* to these progenitors play in defining the outcome of *H. pylori* infection? These investigators have developed a transgenic mouse (tox176) where ablation of parietal cells leads to progressive amplification of these progenitors. The progenitors produce NeuAcalpha2,3Galbeta 1,4-glycans that serve as receptors for *H. pylori* adhesins in vivo. These glycans are also found in human gastric Ca and its precursors. The results suggest that when the relationship between host and *H. pylori* results in loss of parietal cells, as in chronic atrophic gastritis (CAG), *H. pylori* tropism to lineage progenitors may occur if there is a matching of *H. pylori* adhesin and host receptor production. Binding to amplified progenitor cells may then help facilitate initiation and/or progression of tumorigenesis. The investigators will explore this hypothesis using gnotobiotic tox176 mice and test its clinical relevance using materials from a completed Swedish case control study of *H. pylori*-infected patients with Ca + CAG. There are 3 related aims: 1). Obtain a molecular signature of lineage progenitors and of parietal cells. Identify parietal cell factors that affect the progenitors. Lectin panning will be used to recover these cells. Cellular RNA will be probed with Affymetrix GeneChips to identify (i) a panel of molecular markers of these cell types; and (ii) genes expressed in parietal cells that may affect the proliferative status/census of progenitors. The effects of candidates selected from (ii) will be tested directly by gene knockout. 2). Determine the molecular responses of the host when *H. pylori* interacts with NeuAc-alpha2,3Gal-beta1,4+ progenitors. Germ-free normal and tox176 mice will be colonized with an *H. pylori* isolate that produces adhesins that bind to NeuAc-alpha2,3Gal-beta1,4+ progenitors. GeneChips will be used to profile host gene expression before and after colonization. The role of mucosal immune cells in the host response will be examined by gene expression profiling of gnotobiotic Rag1-/normal + tox176 mice. The impact of manipulating expression of the adhesin responsible for progenitor cell attachment will be tested. 3). *H. pylori* isolates from the case control study will be used to characterize *H. pylori* genes that affect host responses. Isolates will be tested for their binding to NeuAc-alpha2,3Gal-beta 1,4 glycans. Whole genome genotyping of binding isolates from cases with Ca + CAG, and from controls + CAG will be performed using DNA arrays containing 1661 amplified OREs from the sequenced *H. pylori* 26695 and J99 strains. Isolates with representative genotypes associated with CAG and Ca will be selected from the panel (or genetically engineered), introduced into tox176 mice, and host responses defined. These studies should yield new insights about *H. pylori* pathogenesis plus markers for identifying patients at risk for severe pathology.

Grant: 1R01DK058587-01A1
Program Director: HAMILTON, FRANK A.
Principal Investigator: PEEK, RICHARD M MD
Title: Helicobacter pylori and gastrointestinal biology
Institution: VANDERBILT UNIVERSITY NASHVILLE, TN
Project Period: 2001/08/01-2006/06/30

DESCRIPTION (provided by applicant): Persistent *H. pylori* infection is a risk factor for atrophic gastritis and distal gastric adenocarcinoma; however, only a small percentage of colonized persons develop neoplasia. Enhanced cancer risk may be related to differences in expression of specific bacterial products, to differences in host response to the bacteria, or to the specific interactions between host and microbe. *H. pylori* strains that possess the *cag* pathogenicity island induce more severe gastritis and are associated with an additional risk for developing atrophy and gastric cancer. A specific mechanism by which *cagA*⁺ strains may lower the threshold for carcinogenesis is by altering epithelial cell proliferation and apoptosis, processes that can be regulated by host inflammatory mediators such as prostaglandin products of cyclooxygenase-2 (COX-2). Over-expression of COX-2 in vitro inhibits apoptosis, and COX-2 is up-regulated within *H. pylori*-induced gastritis, atrophic gastritis, and gastric adenocarcinoma specimens. In vitro, *H. pylori* *cagA*⁺ strains stimulate COX-2 expression in gastric epithelial cells. Since we and others have shown that *cagA*⁺ strains are associated with increased gastric epithelial cell proliferation but attenuated apoptosis in vivo, induction of COX-2 by strain-specific microbial factors may represent a specific mechanism by which certain *H. pylori* strains heighten the risk for gastric adenocarcinoma. The long-term objective of this proposal is to examine the molecular mechanisms by which *H. pylori* strains selectively affect COX-2 regulated epithelial cellular turnover in vitro and in vivo. To address this, we will first determine whether *H. pylori* or secreted bacterial products alter COX-2-dependent apoptosis in a novel in vitro model of bacterial:gastric epithelial cell interaction (conditionally immortalized gastric epithelial cells). COX-2 expression will also be examined in myofibroblasts co-cultured with *H. pylori* and epithelial cells to more closely approximate events occurring within native gastric mucosa. Second, we will determine whether *H. pylori* infection affects COX-2-dependent cellular turnover in wild-type and COX-2 deficient mice. Third, we will investigate the role of specific *H. pylori* determinants on COX-2-regulated cellular responses by inactivating strain-specific genes identified by *H. pylori* whole genome microarray. *H. pylori* parental and isogenic mutant strains will then be co-incubated with conditionally immortalized cells and infected into mice. The effects of strain-specific bacterial factors and COX-2 generated products also will be investigated in a murine model of gastric carcinogenesis, INS-GAS hypergastrinemic mice. Systematic studies of each of these variables in vitro and in animal systems that reflect *H. pylori* pathogenesis in humans should help elucidate their relative importance, direct the course of future intervention and prevention strategies, and potentially provide a model of carcinogenesis arising within the context of chronic mucosal inflammation.

Grant: 1R01DK058911-01A1
Program Director: HAMILTON, FRANK A.
Principal Investigator: KONKEL, MICHAEL E PHD
Title: Pathogenesis of Campylobacter enteritis:
Institution: WASHINGTON STATE UNIVERSITY PULLMAN, WA
Project Period: 2001/08/01-2006/05/31

DESCRIPTION (provided by the applicant): The ultimate goal of this research is to use the knowledge gained from the identification and characterization of Campylobacter jejuni virulence determinants to reduce morbidity and mortality resulting from C. jejuni infections. C. jejuni is a leading cause of human gastrointestinal disease worldwide, causing approximately 3.5 million cases of diarrheal illness per year in the United States. Infection with C. jejuni is characterized by fever, severe abdominal cramps, and diarrhea containing blood and leukocytes. The dysenteric nature of Campylobacter infection, coupled with experimental evidence, supports the notion that C. jejuni must invade the cells lining the gastrointestinal tract for the development of C. jejuni-mediated enteritis. The focus of this proposal is to identify and functionally characterize the bacterial proteins necessary for C. jejuni internalization. Previous work in my laboratory has revealed that C. jejuni synthesize and secrete proteins upon co-cultivation with mammalian cells. These secreted bacterial proteins have been collectively called Campylobacter invasion antigens (Cia). A mutation in a gene encoding the 73 kDa CiaB secreted protein results in a non-invasive phenotype. I hypothesize that Cia proteins are secreted via a Type III export pathway from C. jejuni. The results of this research will better define the pathogenic mechanisms and virulence determinants of one enteric pathogen, C. jejuni, and will be useful in the development of intervention and control methods to reduce the number of cases of human campylobacteriosis.

Grant: 1R01DK059012-01A1
Program Director: HAMILTON, FRANK A.
Principal Investigator: BOEDEKER, EDGAR C MD
Title: Intervention strategies of hemorrhagic colitis and HUS
Institution: UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD
SCHOOL
Project Period: 2001/08/01-2005/07/31

DESCRIPTION (provided by applicant): The broad aim of this proposal is to develop and utilize new and established animal model of enterohemorrhagic E. coli (EHEC) infection, in rabbits and dogs, to develop therapeutic regimens to prevent and treat EHEC disease. It is well recognized that shiga-toxin- (Stx)-producing strains of E. coli, acquired by ingestion of inadequately cooked meat, or other contaminated foods, cause hemorrhagic colitis, and may induce fatal hemolytic uremic syndrome (HUS). EHEC strains produce potent protein toxins named Shiga-like toxins (Stxs) because of their relatedness to Shiga toxin of Shigella dysenteriae. In addition, most EHEC share the ability to adhere intimately to intestinal epithelial cells by "attaching and effacing" (A/E)(7) mechanisms (Fig.2). Although EHEC attachment mechanisms may directly contribute to diarrheal disease, and may influence toxin delivery, the most severe intestinal and renal manifestations of EHEC infection result from toxin-mediated damage to vascular endothelium, with tissue edema, inflammatory infiltrates, cytokine production and vascular thrombi. At present, only supportive care is available to prevent the development of the severe, and frequently fatal, complications of EHEC infection. Strategies aimed at decreasing the toxin burden and preventing the interaction of Stxs with their endothelial receptors should prevent or ameliorate disease and damage in target organs (gut, CNS and kidney). Interventions developed in animal models can subsequently be applied to the prevention and management of EHEC disease. E. coli strain RDEC-H19A infection of rabbits serves as the established animal model of EHEC disease for the initial intervention studies (Aims 1-4). RDEC-H19A, produced by the transfer of the toxin-converting phage H19A of an O26:H11 EHEC to the rabbit entero-pathogenic E. coli RDEC-1, is an attaching and effacing rabbit pathogen which produces high levels of Shiga-like toxin I (Stx-I), colonizes cecum and colon, and induces intestinal disease in rabbits with pathologic changes resembling human EHEC disease. Specific aims (1-4) of the proposal are to use animal models of EHEC infection to: 1). Test the ability of new toxin-receptor analogs, administered parenterally or enterically to prevent EHEC disease. 2). Further test the ability of passively administered immunoglobulin with anti-toxic activity to prevent EHEC disease. 3). Further examine whether antibiotic therapy has beneficial or harmful effects on the course of disease. 4). Further develop strategies for active immunization against EHEC using the Stx toxins of EHEC. 5). Specific aim 5 is to utilize canine specific A/E strains to produce new STEC capable of infecting dogs, which are susceptible to renal vascular lesions. We will transfer our labeled Stx-1 encoding phage to dog-specific A/E strains of E. coli and test their ability to produce intestinal and renal disease. The clinical studies in dogs will be performed at Kansas State University by Dr. Brad Fenwick who has described the Cutaneous and Renal Glomerular Vasculopathy (CRGV) in greyhound dogs exposed to Stx. 6). Specific Aim 6 is to extend our rabbit and dog models to be able to test similar strategies against EBEC strains expressing Stx-2.

We will label and transfer toxin converting phage encoding Stx-2 to rabbit and dog specific A/E strains.

Includes RPGs (Research Project Grants)
Excludes Clinical Trials

Grant: 1R01DK059655-01
Program Director: HAMILTON, FRANK A.
Principal Investigator: SEARS, CYNTHIA L MD
Title: Pathogenicity of Enterotoxigenic *Bacteriodes Fragilis*
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 2001/09/21-2004/08/31

DESCRIPTION (provided by the applicant): *Bacteroides fragilis* are the leading causes of anaerobic bacteremia and intra-abdominal abscesses. Over the past 15 years, increasing data implicate toxin-secreting strains of *B. fragilis* (termed enterotoxigenic *B. fragilis* or ETBF) as causative agents in diarrheal disease afflicting young children and adults. To date, the available human data on ETBF infection has been limited to studies assessing the epidemiological association of ETBF strains with diarrhea and, more recently, inflammatory bowel disease and bloodstream infections. However, the clinical syndrome(s) associated with intestinal ETBF infections are ill-defined and the impact of these infections on intestinal structure and pathophysiology have yet been investigated. The key virulence factor identified to date for ETBF strains is a secreted heat-labile, ca. 20 kD metalloprotease toxin (*B. fragilis* toxin or BFT). Purified BFT and/or infection with ETBF stimulate secretion, rounding of the intestinal epithelial cells with disruption of cell-to-cell contacts and inflammation in both the small bowel and colon of animals. Similar observations have been accrued when intestinal epithelial cell models are treated with BFT in vitro. In these in vitro models, our data show reduced intestinal epithelial cell monolayer resistance, chloride secretion, inhibition of Na⁺ absorption, and secretion of the pro-inflammatory chemokine, interleukin-8. All of these data are consistent with the hypothesis that ETBF and BFT are causative agents of diarrheal disease. To begin to fill in the gaps in our understanding of ETBF disease, the Specific Aims of this proposal are: 1) to study in-depth the epidemiology of ETBF infections; and 2) to investigate the pathogenesis of ETBF infections with a particular focus on their impact on intestinal structure and function. ETBF will be identified in the stools of children and adults admitted to the diarrhea hospital of the International Centre of Diarrheal Disease Research in Bangladesh where ETBF infection has previously been shown to be significantly associated with diarrheal disease. Clinical data, acute and convalescent serum samples, stool studies to evaluate for evidence of inflammatory mediators and colon biopsies will be obtained and correlated to define the epidemiology and pathophysiology of the infection. We hypothesize that ETBF is an under-recognized intestinal pathogen accounting for a portion of previously undiagnosed inflammatory intestinal infections in both children and adults.

Grant: 1R01DK060061-01
Program Director: HAMILTON, FRANK A.
Principal Investigator: MAIER, ROBERT J BS
Title: Oxidative Stress and Survival of Helicobacter pylori
Institution: UNIVERSITY OF GEORGIA ATHENS, GA
Project Period: 2001/08/01-2006/07/31

DESCRIPTION (provided by the applicant): Helicobacter pylori is a spiral bacterium that colonizes the gastric mucosa of humans, leading to a variety of inflammatory gastric diseases that include peptic ulcers, chronic gastritis, adenocarcinoma of the lower stomach, and MALToma (mucosal-associated lymphoma). The severity of the inflammatory-based disease is related to the persistent nature of the pathogen, and chronic infection is the predominant pre-disposing factor for carcinoma. The persistence is attributed to the pathogens' stringent adaptation to the harsh environment of the human stomach, which must include avoiding acidity and combating host defense mechanisms. The battery of host-produced partially reduced oxygen species and other reactive molecules that damage the bacterial cellular components needed for survival of the pathogen are in turn counteracted by enzymes produced by the successful pathogen. The goal is to identify and characterize the antioxidant enzymes produced by H pylori to combat oxidative stress and maintain virulence (stomach colonization). This will be approached by targeted mutagenesis of five specific antioxidant genes. Then each mutant will be characterized for its ability to withstand oxidative stresses, to acquire spontaneous mutations, to survive air-exposure, and to colonize mouse stomachs. The environmental host-related signals (such as iron, oxygen, mucin, pH, and oxidizing agent) that may regulate each of the five genes will be determined, and some of the antioxidant activities will be characterized. In addition to the five targeted antioxidant activities, global approaches involving proteomics and DNA microarray will be used to determine the number and nature of proteins that are expressed upon adaptation of H pylori to oxidative stress conditions.

Grant: 1R01DK060163-01
Program Director: MULLINS, CHRISTOPHER V.
Principal Investigator: COLLINS, CARLEEN M PHD VET
MEDICINE:MICROBIOLOGY
Title: Urea-dependent virulence in uropathogenic bacteria
Institution: UNIVERSITY OF MIAMI-MEDICAL CORAL GABLES, FL
Project Period: 2001/09/21-2002/06/30

DESCRIPTION(provided by applicant: Urease, which catalyzes the hydrolysis of urea to ammonia and carbonic acid is produced by diverse bacterial species including various aerobes, facultative anaerobes and obligate anaerobes Both Gram-negative and Gram-positive organisms are urease producers, as are species of mycobacteria and ureaplasma. Urease plays a significant role in virulence when expressed by urinary tract, oral, and gastroduodenal pathogens. *Providencia stuartii* and *Proteus mirabilis*, the two most common ureolytic uropathogens, express urease only in the presence of urea. This urea-dependent expression is mediated by UreR, a transcriptional activator belonging to the AraC family of regulators. Evidence suggests that urea interacts directly with UreR, and thus is the effector molecule for this activator. Urea is found at concentrations up to 500 mM in the urinary tract, a concentration that is at least 50 fold higher than that observed at other sites in the body. Thus for these uropathogens, urea is a signal molecule, and UreR is acting as a signal receptor, alerting the organism that it is in the urinary tract. UreR bound to urea is active as a transcriptional activator and has a high affinity for DNA. UreR not bound to urea is not active and has a low affinity for the DNA binding site. Studies in this proposal are to examine the urea-UreR interaction and to determine the conformational changes associated with urea binding that result in active UreR. Two models are proposed, one in which UreR forms a dimer, and the other in which UreR is active as a monomer. Studies are proposed to prove one of these models. The crucial urea-UreR interaction is examined in Aim #1, and the urea-UreR-DNA interaction in Aim #2. Aim #3 is to determine the X-ray structure of UreR, UreR bound to urea, and UreR bound to urea and the DNA binding site. Structures of mutant forms of UreR will also be generated. This work will elucidate the molecular mechanisms of this important regulator of uropathogen virulence, as well as extend our knowledge on the AraC family of transcriptional activators.

Grant: 1R01DK061769-01
Program Director: HAMILTON, FRANK A.
Principal Investigator: CROWE, SHEILA E MD
Title: Oxidative damage to gastric epithelial cells by H pylori
Institution: UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA
CHARLOTTESVILLE
Project Period: 2001/09/21-2006/08/31

Helicobacter pylori is a chronic infection that affects 50% of the world's population causing gastritis in all infected while only a subset develop disease of the epithelium in the form of ulceration or adenocarcinoma. Both bacterial and host factors appear to play a role in the pathogenesis of these human diseases but the specific mechanisms remain unclear. *H. pylori* and cytokines known to be increased in *H. pylori* infection, induce alterations of gastric epithelial cell growth such as the induction of programmed cell death. Phagocytic leukocytes recruited to the gastric mucosa during infection become activated, generating reactive oxygen species (ROS) that we have shown to alter gastric epithelial cell growth and induce apoptosis. Infection with *H. pylori* also induces the accumulation of ROS in gastric epithelial cells that may be dependent on bacterial genotype. Gastric epithelial cells respond to oxidative stress with the initial generation of ROS and subsequent activation of a redox-sensitive signaling pathway which has been shown to control the transcription of genes that regulate cell growth, repair and death processes. Of particular interest is ROS-induced activation of apurinic/apyrimidinic endonuclease-1 (AP endonuclease), a multifunctional protein that is the rate-limiting enzyme in the DNA base excision repair pathway of oxidative lesions, which also activates transcription factors including activator protein (AP)-1, and p53. Thus, the general hypothesis underlying this proposal is that oxidative stress contributes to the epithelial cell injury that occurs during *H. pylori* infection. The specific hypothesis that *H. pylori* infection stimulates redox-sensitive signaling through AP endonuclease that leads to apoptosis in gastric epithelial cells will be examined in the following specific aims: Aim 1. Evaluate oxidative stress in gastric epithelial cell injury (apoptosis) during *H. pylori* infection; Aim 2 Determine if *H. pylori* regulates the expression and function of AP endonuclease in gastric epithelial cells; Aim 3. Define how AP endonuclease regulates apoptosis and the transcription of pro-apoptotic genes. These studies in cultured human cell lines and human tissue will address unanswered questions regarding the effect of oxidative stress on gastric epithelial cell injury. The molecular mechanisms governing the epithelial response to oxidative stress will also be defined. This new knowledge will improve our understanding of the pathogenesis of epithelial cell damage associated with *H. pylori* infection and help identify strategies for the prevention and treatment of human gastric disease.

Grant: 1R03DK056664-01A2
Program Director: HAMILTON, FRANK A.
Principal Investigator: ASHKTORAB, HASSAN PHD
Title: Mechanisms of Growth Inhibition by Helicobacter Pylori
Institution: HOWARD UNIVERSITY WASHINGTON, DC
Project Period: 2001/09/30-2003/08/31

DESCRIPTION (provided by applicant): The discovery that H. pylori is an important factor in the development of peptic ulcers has dramatically changed the way ulcer patients are treated. Ulcers heal faster in persons treated with antibiotics in addition to acid medication. One possible explanation for this observation is that cell generation is impaired by H. pylori; thus, ulcer healing occurs more rapidly in the absence of H. pylori infection. Epidemiological studies have strongly associated H. pylori with gastric carcinogenesis. These data led the World Health Organization to designate H. pylori a Class I carcinogen. It is felt that at least half of all gastric cancers are attributed to infection with this bacterium. However, there is little known as to how H. pylori may directly effect gastric cells to cause gastric cancer. Epidemiological data supports this bacterium as a cofactor because it causes chronic gastritis which may progress to atrophic gastritis, a precursor lesion for intestinal type gastric cancer. However, this bacterium is also strongly linked to diffuse gastric cancer which occurs in otherwise normal (non-atrophic) gastric mucosa, where the bacterial infection is present at the time the cancer occurs. In vivo studies show that some bacterial strains cause significant cell injury in the absence of a rise in gastric apoptosis. One explanation is that the bacterium, while causing cell injury, is able to down regulate apoptosis. The decrease in apoptosis in injured gastric cells is one possible mechanism by which this bacterium might directly increase the susceptibility of gastric cells to carcinogenic conversion. This grant proposes to evaluate the direct effects of H. pylori on the cell death in gastric epithelial cells. The Specific Aims of this project are: 1) To better elucidate the involvement of the p53 pathway in H. pylori induced apoptosis by determining the extent of phosphorylation of p53 in cells exposed to H. pylori strains and the importance of phosphorylation at serine-15, -20 and -46 in regard to apoptosis. Also, to evaluate p53 phosphorylation in response to ROS species in the absence of H. pylori and in the presence of H. pylori and antioxidants. 2) To determine the involvement and significance of activation of p53AIP1, which is activated by phosphorylating p53 at serine-46. The mechanism of bacterial exposure on the gastric epithelial cell death has not been studied in regard to p53. These studies are important to elucidate specific p53 pathways that are modulated by exposure to this bacterium, resulting in an regulating cell death. Specifically, whether or not stimulation of reactive oxygen species (ROS) by H. pylori is essential for p53 phosphorylation leading to apoptosis. These studies will help to establish how bacteria can increase apoptosis possibly through generation of ROS and in specific circumstances this may alter one's risk of developing cancer.

Grant: 1R03DK058576-01
Program Director: BISHOP, TERRY ROGERS
Principal Investigator: GOLUSZKO, PAWEL PHD
Title: INTERLEUKIN-12 REGULATION BY DR-FIMBRIATED E. COLI
Institution: UNIVERSITY OF TEXAS MEDICAL BR GALVESTON, TX
GALVESTON
Project Period: 2001/06/01-2003/05/31

DESCRIPTION (Adapted from the Applicant's Abstract): The mechanism of recurrent urinary tract infection (UTI) and its effect on the kidney function is not well understood. The persistence is a characteristic feature of UTI caused by E. coli that express Dr fimbriae. Uropathogenic E. coli strains bearing Dr fimbriae recognize complement regulatory protein-decay accelerating factor (DAF) as their receptor. Interaction of Dr fimbriae with DAF is associated with cross-linking of receptor, followed by internalization of Dr-positive E. coli into epithelial cells. DAF is a surface protein anchored in the membrane via the glycolipid glycosylphosphatidylinositol (GPI). Recent findings indicate that cross-linking of complement regulatory proteins on human monocytes/macrophages by certain intracellular pathogens inhibits the production of key immunoregulatory cytokine, interleukin-12 (IL-12). This finding raises the possibility that Dr-positive E. coli may alter cell immune response by downregulating IL-12, followed by impaired production of interferon , a potent stimulator of bactericidal activity of macrophages. The investigators propose to evaluate in three Specific Aims the general hypothesis that cross-linking of DAF on human monocytes/macrophages by E. coli bearing Dr fimbriae inhibits IL-2 production.

Grant: 1R03DK058666-01A1
Program Director: PODSKALNY, JUDITH M.
Principal Investigator: DIELEMAN, LEVINUS A MD
Title: Mechanisms of bacterial-induced colitis in HLA-B27 rats
Institution: UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC
HILL
Project Period: 2001/09/10-2003/06/30

DESCRIPTION (provided by applicant) Rodent models of chronic intestinal inflammation have contributed substantially to our knowledge of the pathogenesis of IBD. One better characterized model is HLA-B27 transgenic rats, in which the overexpression of the gene for the MHC class I molecule HLA-B27 leads to the spontaneous development of colitis, gastroduodenitis, peripheral arthritis and spondylitis. Our hypothesis is that chronic colitis and gastritis is the result of an overly aggressive immune response to luminal bacteria in a genetically susceptible host. This T lymphocyte-dominated immune response to specific luminal bacteria is regulated by antigen presenting cells (APC). This hypothesis is evaluated in HLA-B27 transgenic rats, which develop progressive colitis, gastritis and arthritis when raised in specific pathogen-free environment or when colonized with *Bacteroides vulgatus*, but which have no clinical or histological disease when raised in a sterile environment or monoassociated with *E. coli*. In the first 2 years of the K08 award (DK 02551) we studied the role of inducing and protective intestinal organisms in our model. In specific-pathogen-free conditions (SPF) we found that oral broad spectrum antibiotics can prevent and treat established colitis in SPF B27 TG rats, which will recur after the treatment is stopped. *Lactobacillus casei* GG (L.GG) can prevent the disease recurrence after treatment with antibiotics. However, the immunological mechanisms determining how these bacteria prevent a chronic immune response need to be elucidated. In this R03 application we will address the following specific aims: 1. Determine in-vivo immune mechanisms by which *Lactobacillus* GG can prevent relapse of colitis in SPF HLA-B27 TG rats treated with antibiotics. 2. Investigate if protective immune responses are mediated by L.GG-specific inhibitory T lymphocytes.

Grant: 1R21DK060818-01
Program Director: SCHERBENSKE, M. JAMES
Principal Investigator: BLOUNT, PAUL L PHD PHYSIOLOGY, OTHER
Title: Using Microbial Genetics to Study Eukaryotic Channels
Institution: UNIVERSITY OF TEXAS SW MED DALLAS, TX
CTR/DALLAS
Project Period: 2001/09/30-2003/08/31

DESCRIPTION(provided by applicant): The ability to detect mechanical and thermal stimuli is at the foundation of many life-sustaining systems including renal function as well as cardiovascular and thermal regulation. In addition, mechanosensation is the necessary primary event for the senses of hearing, balance, touch and pain. However, little is known about the molecular mechanisms underlying these senses. The best candidates for sensors at the base of these phenomena in mammalian systems are ion channels that have only recently been discovered. Previously, researchers have demonstrated heterologous functional expression of many eukaryotic transporters and channels in bacteria and yeast. Given these numerous examples, we anticipate that some of the newly discovered candidate mechano- and thermo-sensitive channels may be functionally expressed in microbial systems. Microbial heterologous expression has the unique advantage that the expressed gene can be randomly mutated and rare mutational events rapidly screened or selected. In this way, structural changes can be correlated with functional differences, thus giving insight into the molecular mechanisms of the protein. Here we propose to utilize the power of microbial genetics to study the structure-function relationships of eukaryotic channels which are thought to gate in response to mechanical and/or thermal stimuli. Previously, it was demonstrated that *E. coli* strains deficient in mechanosensitive channels have an osmotic-dependent cell death phenotype. Candidate mammalian sensors will be heterologously expressed in one of these strains and their ability to suppress this phenotype assayed. Several candidates will be examined and the one(s) that give the most promising results will be aggressively pursued. Mutations that evoke improved suppression, slowed growth or flux-dependent phenotypes will be determined. All resulting mutants will be assayed for osmotic-dependent ion fluxes and channel activity to allow for the correlation of structural with functional changes. Finally, we will pursue the development of a system that will allow for a similar study of homologously and heterologously expressed mechano- and thermo-sensitive eukaryotic channels in yeast (*S. pombe*).

Grant: 1R21DK060841-01
Program Director: SECHI, SALVATORE
Principal Investigator: WANG, DA-NENG PHD
Title: Overexpression/crystallization of amino acid transporter
Institution: NEW YORK UNIVERSITY SCHOOL OF NEW YORK, NY
MEDICINE
Project Period: 2001/09/30-2003/08/31

DESCRIPTION (provided by applicant): Amino acids are essential building blocks for proteins and many other molecules in the cell. In the central nervous system, they serve as major signaling molecules. Amino acid uptake and secretion across the cell membrane are mediated by a group of membrane proteins, called amino acid transporters. These transporters are involved in many important physiological processes, including: nutrient uptake at the absorptive epithelia in the small intestine, transport at the kidney proximal tubule for glucogenesis and hepatic regeneration, supply of amino acids to the fetus by the mother at the placenta, and chemical neurotransmission at the synapse. Equally important, amino acid transporters are associated with numerous pathological conditions, such as cystinuria, lysinuria, schizophrenia, ischemia and cocaine addiction. To understand the molecular mechanism of amino acid transporters, three-dimensional structural information generated by crystallography is needed. Transporters from mammalian organisms are currently not suitable for crystallization, due to their low natural abundance and lack of appropriate overexpression systems. Bacterial amino acid transporters share sequence homology with their mammalian counterparts, and thus most likely have similar three-dimensional structures. We therefore plan to overexpress, purify and crystallize bacterial amino acid transporters from the amino acid/polyamine/choline transporter family. Proteins will be overexpressed in *E. coli* and purified to homogeneity. Crystallization strategy begins with screening homologues from different species and protein modification by genetic techniques. Crystal nucleation will be screened with various polyethylene glycols and pHs. Crystalline order will be improved by adjusting detergent micellar size and co-crystallization with inhibitors and heavy metal salts. Well-ordered crystals will enable us to determine the transporter structure at atomic resolution later. The structure will reveal the amino acid binding pocket and substrate translocation pathway, and will allow us to design experiments to elucidate the roles that critical residues play in the transport process. Such structural information will also shed light on the structure and molecular mechanism for mammalian amino acid transporters.

Grant: 2R01ES004050-16A1
Program Director: THOMPSON, CLAUDIA L
Principal Investigator: CHAKRABARTY, ANANDA M BA
Title: Microbial Degradation of Agent Orange
Institution: UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL
Project Period: 1986/04/01-2005/06/30

Various chemicals are released in our environment as herbicides/pesticides, degreasers, fire retardants, industrial solvents or as by-products of chemical industry, leading to major environmental pollution problem. The U.S. Congress specifically authorized the Super Funds to clean up thousands of toxic dump sites in the United States, but very few have been cleaned up. Microorganisms are responsible for recycling natural wastes, but they are relatively inert towards synthetic compounds, particularly the high chlorinated compounds that occur rarely in the natural environment. Yet, microorganisms are highly adaptable and have evolved, and are continually evolving, the genes, both structural and regulatory, that specify biodegradation of a variety of simple chlorinated compounds. Microbial remediation of highly chlorinated compounds is thus a major goal to address problems of toxic chemical pollution. To accelerate the rate of microbial biodegradation of a toxic, synthetic chemical, one needs to understand the nature of regulation of the structural genes, and the mechanism of action as well as evolution of such regulatory genes. However, even when a microorganism becomes available for effective degradation and removal of a toxic chemical, it cannot necessarily be used in bioremediation unless its pathogenic potential to local populations has been determined. This proposal has two major goals. The first to understand how degradative pathways for simple, as well as somewhat recalcitrant, chlorinated compounds evolve in nature. We are particularly interested in the evolution and the mode of action of regulatory genes that regulate the level of expression of the structural genes in the biodegradative pathway. Our second goal is to develop both conceptual and technical understanding and approaches to assess the pathogenic potential of a bioremediating organism. The progress achieved and the ways we intend to meet our goals are detailed in this proposal

Grant: 1R01ES010793-01A1
Program Director: LAWLER, CINDY P
Principal Investigator: LEWITT, PETER A MD OTHER AREAS
Title: Nocardia: A Novel Environmental Agent For Parkinsonism?
Institution: WILLIAM BEAUMONT HOSPITAL RESEARCH ROYAL OAK, MI
INST
Project Period: 2001/08/01-2004/07/31

DESCRIPTION (Adapted from applicant's abstract): The acid-fast bacterium *Nocardia asteroides* (NA) is widespread in the environment, and subclinical human infection is common. Experimental infection of mice with this organism results in loss of nigrostriatal dopaminergic neurons, decreased striatal dopamine concentration, and movement abnormalities including head shaking and slowness of movement. These neurochemical and motor alterations are similar to those in Parkinson's disease (PD). The long range goal of our studies is to determine how and why neurons in the substantia nigra pars compacta (SNpc) deteriorate in PD. The objective of this application is to determine the relevance of NA infection as an animal model for PD and a potential causative agent of PD. Our central hypothesis is that CNS NA infection results in the specific loss of dopaminergic neurons and development of motor abnormalities by mechanisms similar to those suggested for PD. This hypothesis has been formulated on the basis of strong preliminary data, suggesting that (a) nocardial toxicity in the SNpc is dopamine neuron-specific, (b) histological findings, including dopamine neuron loss and development of Lewy body-like inclusions, are similar to those in PD, and (c) some of the movement abnormalities in these animals result from striatal dopamine depletion, because they respond to levodopa administration. The rationale for this research is that an understanding of the process by which experimental NA infection causes loss of dopaminergic neurons and motor abnormalities may enhance our ability to prevent this process in PD. *Nocardia* may be a cause of PD because the organism can survive long-term in the brain as an L-form, suggesting that chronic, subclinical infection could result in neurodegeneration. We are uniquely qualified to perform this research because of our experience with the various in vivo and in vitro aspects of this model. The central hypothesis will be tested and the objective of the application accomplished by pursuing three specific aims: (1) Determine the relationship between neurological and motor abnormalities in mice experimentally infected with NA, and the relevance of this model to PD, (2) Characterize the specificity and mechanism of NA toxicity, and (3) Determine whether evidence for NA infection can be detected in brain specimens from NA-infected mice and from human subjects with PD and Parkinsonian-like syndromes. This work is innovative because it explores the novel hypothesis that an infectious agent may offer a new model for PD and may even contribute to the disease. It is our expectation that these studies will reveal the extent to which this animal model resembles PD, as well as whether persistent NA infection occurs in the PD brain. These outcomes will be significant because they should add to our understanding of the neurodegenerative process in PD, and could lead to improved means of diagnosis and treatment for individuals with this disorder.

Grant: 1R01ES011682-01
Program Director: COLLMAN, GWEN
Principal Investigator: WANG, XIAOBIN MD
Title: DDT, Endocrine Disruption and Reproductive Outcomes
Institution: BOSTON MEDICAL CENTER BOSTON, MA
Project Period: 2001/09/01-2004/08/31

DESCRIPTION: This proposal responds to RFA OH-01-001 "Endocrine Disruptors: Epidemiologic Approaches." Both animal and in vitro studies support the hypothesis that dichlorodiphenyl trichloroethane (DDT) and its metabolites are potentially important human reproductive toxins. However, the epidemiologic data associating DDT with human reproductive health are limited. The goal of this proposal is to establish a dose-response relationship between exposure to dichlorodiphenyl dichloroethene (DDE), a major and stable metabolite of DDT, endocrine dysfunction, and adverse reproductive outcomes in women. We will test the hypotheses that exposure to DDE is associated with (1) hormone dysfunction including reduced estrogen excretion (REE), anovulation, abnormal luteal phase (ALP), and abnormal follicular phase (AFP); (2) menstrual disorders; (3) reduced fecundability; and (4) adverse pregnancy outcomes including spontaneous abortion (SAB), preterm delivery, low birth weight, and intrauterine growth retardation (IUGR). We will further test the hypotheses that endocrine disruption evaluated by REE, anovulation, ALP, and AFP is associated with above adverse reproductive outcomes. This proposal is built on a large prospective cohort study in Anqing, China, funded by NICHD (HD32505, period: 1996-2001) to evaluate the effects of rotating shift work on reproductive outcomes. A total of 1,200 married women employees of Anqing Textile Mill who were between 20 and 34 years of age, never smokers, and who obtained permission to have a child and attempted to become pregnant over the course of the study have been enrolled in the parent study. Information available from this cohort includes: (1) detailed baseline and follow-up questionnaires on sociodemographic characteristics, reproductive history, occupational and environmental factors, and dietary intake; (2) daily diary from each woman reporting menstruation, use of medications, sexual intercourse, contraceptive use, active and passive smoking, alcohol use, and occupational exposure; (3) clinical data on reproductive outcomes, including menstrual disturbances, time to conception, SAB, preterm delivery, low birth weight, and IUGR; (4) daily urine samples from each woman for up to one year or until pregnancy is clinically confirmed; (5) measures of time to conception and subclinical fetal loss determined by a highly sensitive and specific assay for urinary B-hCG; and (6) archived pre-pregnant plasma samples from each woman. This proposal will include the 1,200 women already enrolled in the parent study. Plasma DDT/DDE levels will be measured and urinary pregnanediol-3-glucuronide (PdG), estrogen conjugates (E1C), and follicle stimulating hormone (FSH) will be analyzed. These newly obtained pesticide and hormone data will be linked to existent epidemiologic and clinical database to investigate dose-response relationships between DDE exposure, hormone dysfunction, and adverse reproductive outcomes, with adjustment for important confounders.

Grant: 1R01ES011683-01
Program Director: COLLMAN, GWEN
Principal Investigator: RAYMER, JAMES H PHD
Title: Epidemiologic Evaluation of Perfluorooctyl Compounds
Institution: RESEARCH TRIANGLE INSTITUTE RESEARCH TRIANGLE PARK
NC
Project Period: 2001/09/01-2004/08/31

DESCRIPTION: This epidemiologic study will evaluate the potential endocrine and reproductive ramifications in human males of exposures to environmental concentrations of perfluorinated chemicals, including perfluorooctylsulfonate (PFOS) and perfluorooctanoate (PFOA). Products containing these chemicals were recently withdrawn from the market by a major manufacturer amid concerns of persistence, toxicity, and widespread population exposures to these chemicals. The proposed study will focus on the semen quality and endocrine status of a potentially susceptible subpopulation, i.e., men of couples who present at a fertility clinic. It will use a case-control design in that the study population will include a high prevalence of men experiencing reproductive problems ("cases") as well as men with a normal fertility status ("controls"). By using a case-control design (the approach of choice for investigating rare outcomes), the study will be able to efficiently detect any important exposure-related reproductive problems. If exposure levels are associated with male reproductive problems, the study participants are expected to represent a range of exposure to PFOA and PFOS, which will be measured in samples of blood and semen; concentrations in these biological media will reflect the multi-route exposures to these chemicals experienced by virtually all people in our society. Semen quality will be assessed using both routine measures and a test designed to more accurately and reproducibly assess normal, motile, and fertile sperm. Measurements of Follicle Stimulating Hormone, Luteinizing Hormone, Prolactin, Estradiol and free and total Testosterone will reflect the hormonal status of the males and will provide evidence of perturbed endocrine function. If the exposure effect is limited to a sensitive subset of the general population, our study is more likely to detect an association compared to studies that sample on PFOA/PFOS exposure status in an occupational setting.

Grant: 1R15ES010964-01
Program Director: WEIS, BRENDA K
Principal Investigator: YATES, JAMES R PHD
Title: Gene regulation of the bph cluster
Institution: UNIVERSITY OF SOUTH CAROLINA AT COLUMBIA, SC
AIKEN
Project Period: 2001/04/03-2005/03/31

DESCRIPTION: (Provided by applicant): It is estimated that up to 20 percent of the 1.4 billion lbs. of Polychlorinated biphenyls (PCBs) produced from 1929 to 1978 have entered the environment. These pollutants pose a serious threat to human health. It appears that the most reasonable and cost efficient method of removing these compounds is via biological means. The ubiquitous nature and vast biochemical capabilities of bacteria make them the organisms of choice for bioremediation of PCBs. A group of bacteria composed of many different species contains a nearly identical suite of genes (called the bph cluster) encoding enzymes for PCB degradation. The organization and structure of the bph cluster is understood at a rudimentary level, but it is essential that the regulation of these genes be investigated. It is reasonable to assume that an understanding of bph cluster gene regulation will significantly enhance the usefulness of these organisms for solving the environmental PCB problem. The first specific aim of this proposal will be to characterize and clone the entire bph cluster. Although portion of the bph cluster from a Burkholderia sp. called LB400 has been cloned, it is not complete. We will complete the cloning of the bph_cluster and identify all the genes present. The second specific aim is to identify promoters for all of these genes. Previous work has shown that multiple promoters are present and some may be up-regulated when biphenyl is present. However, this study only investigated promoters from one region of the bph cluster. We will propose to complete this promoter study for all of the genes present. The final specific aim is to identify regulatory mechanisms that control the expression of bph cluster genes. This information is absolutely essential for a complete understanding of how PCBs are removed from the environment, and may be useful as a means of enhancing the biodegradative rates of bacteria. The persistence of PCBs in the ecosystem as well as the known health risks to humans clearly justify the proposed research project.

Grant: 1U19ES011375-01
Program Director: MCCLURE, MICHAEL
Principal Investigator: SCHWARTZ, DAVID A
Title: CENTER FOR ENVIRONMENTAL GENOMICS
Institution: DUKE UNIVERSITY DURHAM, NC
Project Period: 2001/09/25-2006/08/31

DESCRIPTION (provided by applicant) Despite the tremendous inter-individual variability in the respond to environmental toxins, the investigators simply do not understand why certain people develop disease when challenged with environmental agents and others remain healthy. Yet, there is emerging consensus that many of the complex (and prevalent) diseases that humans develop occur as a result of multiple biologically unique gene-gene and gene-environment interactions. The recent advances in human and molecular genetics has provided an unparalleled opportunity to understand how genes and genetic changes interact with environmental stimuli to either preserve health or cause disease. The theme of this Center is to use gene expression profiling to understand the effect of environmental stresses on human health. This will be accomplished by establishing an interdisciplinary Center that supports the use of complementary biologic systems (humans, mice, zebrafish and worms) to investigate the role of genetic susceptibility in the pathogenic response to specific types of environmental stress (bacteria, malnutrition, and metals). This approach will enable the investigators to develop and investigate environmental models of human disease that represent biologically unique gene-environment- pathophysiological phenotypes. Microarray analyses will be used to comprehensively evaluate the biological response to environmental stress and to identify pathogenic mechanisms that are relevant to innate immunity, neural tube defects, and transition metal toxicity. The end result is a broad based yet highly integrated program that has the potential to make a number of novel, related observations. The overall hypothesis unifying this research program is that gene expression profiling will identify genes and pathogenic processes that are critical to human environmental health and disease. In aggregates the coupled scientific findings from the proposed Program will substantially enhance our understanding of environmental toxicology and genomics.

Grant: 2R01EY008289-13
Program Director: DUDLEY, PETER A
Principal Investigator: GILMORE, MICHAEL S AB
Title: Roles of Toxins in Endophthalmitis
Institution: UNIVERSITY OF OKLAHOMA HLTH SCIENCES CTR OKLAHOMA CITY, OK
Project Period: 1989/08/01-2006/09/29

DESCRIPTION (provided by applicant): Endophthalmitis is one of the most sight-threatening complications of ocular surgery or penetrating injury. Despite aggressive therapeutic and surgical intervention, endophthalmitis frequently results in partial to complete loss of vision, often within a few days of infection. The overarching goal of this research is to develop a scientific basis for management of endophthalmitis, based on the optimized use of antimicrobial and anti-inflammatory agents, toward enhancing the recovery of useful vision. The following specific aims of the proposed 5 year continuation period are designed, based on the results from the previous period of support, to advance this goal: Specific Aim 1: Determine the molecular basis of virulence for the most common etiology of endophthalmitis associated with visual loss. Specific Aim 2: Develop a murine model system that permits complete analysis of host and bacterial factors that contribute to the pathogenesis of endophthalmitis, and determine the basis for 1) differences in pathogenesis of endophthalmitis due to virulent and avirulent organisms, 2) differences in the pathogenesis of endophthalmitis when infection is acquired by anterior and posterior routes, and 3) the contribution of immune privilege to the pathogenesis of endophthalmitis and mechanisms by which this may be undermined by specific pathogens. Specific Aim 3: Resolve the current controversy surrounding the use of anti-inflammatory adjunctive therapies in endophthalmitis management, and test new specifically targeted therapies for their value in mitigating visual loss.

Grant: 1R01EY013175-01A1
Program Director: FISHER, RICHARD S
Principal Investigator: MCDERMOTT, ALISON M PHD
Title: Defensins and Corneal Wound Healing
Institution: UNIVERSITY OF HOUSTON HOUSTON, TX
Project Period: 2001/08/01-2004/07/31

DESCRIPTION (provided by applicant): Damage to the cornea compromises its important optical and protective functions; rapid repair is therefore critical. However, the mechanisms and factors orchestrating wound healing are unclear. A better understanding of the process is essential to our ability to improve and regulate corneal repair after accidental or surgical injury and in diseases such as recurrent erosion syndrome and diabetes where the integrity of the cornea is compromised. The overall goal of this project is to determine if and how defensins participate in corneal wound healing. Defensins are peptides best known for their antimicrobial activity but whose functional repertoire also includes mitogenic, chemoattractant and signaling properties. Two types of defensin are available to the cornea, alpha-defensins are present in tears and neutrophils recruited after injury and beta-defensins are produced in situ by corneal epithelial cells. Our overlying hypothesis is that alpha and beta defensins promote corneal wound healing. We hypothesize that they do so by stimulating corneal epithelial cell and fibroblast migration, proliferation and production of cytokines/growth factors. Furthermore, we hypothesize that cytokines produced after epithelial injury upregulate the expression of endogenous epithelial defensins which are then available in sufficient quantities to exert a physiological effect. Human corneal epithelial cells and fibroblasts and human corneas in organ culture will be utilized to address the following hypotheses: Specific Aim 1: Hypothesis: alpha and beta defensins stimulate human corneal epithelial cell and fibroblast migration and proliferation. Specific Aim 2: Hypothesis: alpha and beta defensins upregulate the production of cytokines and growth factors by human corneal epithelial cells and fibroblasts. Specific Aim 3: Hypothesis: Expression of endogenous beta-defensin 2 is upregulated after injury in response to an increase in cytokines. Specific Aim 4: Hypothesis: alpha and beta defensins stimulate re-epithelialization in wounded human corneas. The results of these studies will help delineate the as yet unidentified role of defensins in corneal wound healing and restoration of normal corneal function.

Grant: 1R03EY013779-01
Program Director: FISHER, RICHARD S
Principal Investigator: PHILLIPS, THOMAS E BS
Title: MU cells in mammalian conjunctiva
Institution: UNIVERSITY OF MISSOURI COLUMBIA COLUMBIA, MO
Project Period: 2001/09/01-2004/09/01

DESCRIPTION: (Applicant's Abstract) M cells (membranous epithelial cells) are essential participants in immunological receptivity phenomena of most mucosae. Cells with the distinctive morphological and physiological phenotype of M cells have been found in the epithelium overlying mucosa-associated lymphoid tissue in the intestine, bronchi, nasal cavity, and tonsils. M cells initiate mucosal immune events by preferentially binding and translocating soluble and particulate antigens across the surface epithelium and delivering captured antigens to underlying antigenpresenting cells. The follicle-associated epithelium in the mammalian conjunctiva has cells with striking morphological similarity to intestinal M cells but a lack of data on the ability of these cells to transport antigens or pathogens has led some investigators to question their existence in the conjunctiva. We have identified an innovative approach in which an attenuated *Shigella* strain's natural ability to selectively bind and translocate across M cells can be used to unequivocally demonstrate that the conjunctiva contains fully functional M cells. Multiple species will be examined to establish the ubiquity of M cells in the mammalian conjunctiva. The effect of aging on M cell morphology and function will be also investigated. We will seek to demonstrate ocular immunization is optimal for generating local mucosal immunity by comparing the immune response to ocular topical and intra-nasal immunization with an M-cell targeted immunogen. Demonstration that there are M cells capable of antigen sampling in the conjunctiva will link ocular immunology to the growing body of research concerned with targeted mucosal vaccines. Our long-term goals include designing M-cell targeted vaccines to develop ocular immunity against common ocular pathogens such as *Chlamydia trachomatis*. Recognition of conjunctival M cells would also lay the foundation for future studies examining whether opportunistic bacterial or viral pathogens use the M cell as an entry site to cross the mucosal barrier in the eye.

Grant: 2P01GM022778-26
Program Director: LEWIS, CATHERINE D.
Principal Investigator: MOORE, PETER B
Title: PROGRAM IN MACROMOLECULAR STRUCTURE, MOTION, CONTROL
Institution: YALE UNIVERSITY NEW HAVEN, CT
Project Period: 1976/04/01-2006/03/31

This Program supports the study of macromolecules, such as catalytic RNAs, and macromolecular assemblies like the ribosome and the plasma membrane. The RNAs, ribonucleoproteins, and enzymes responsible for gene expression, the proteins that regulate expression, and membrane proteins will be emphasized over the next five years. The primary techniques used to characterize these molecules will be single-crystal X-ray diffraction, where possible, and X-ray scattering and high-resolution cryoelectron microscopy when only solutions or partially ordered samples are available. This Program will also support studies of the motions that occur both within biological macromolecules and between components of macromolecular assemblies as they function. Motion can be inferred from conformational differences observed when time average structures are determined for a given macromolecule under different environmental conditions or in different states of ligation. Of special interest are the motions that occur: (1) during the catalytic cycles of RNA and DNA polymerases and other enzymes, (2) in the course of protein synthesis as the ribosome proceeds through its elongation cycle, and (3) during the insertion of intrinsic membrane proteins into lipid bilayers and the passage of secreted proteins through membranes. Theoretical investigations will also be undertaken of protein-membrane interactions, and work will continue on improving the computational procedures used to obtain structures both by X-ray crystallography and by NMR.

Grant: 2P01GM031299-19
Program Director: LEWIS, CATHERINE D.
Principal Investigator: DICKERSON, RICHARD E
Title: Molecular Basis of Biological Recognition and Assembly
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 1983/04/01-2006/04/30

This Program Project represents an ongoing consortium of eight scientific investigators at UCLA, all of whom work together in using X-ray crystallography, NMR, EPR, or computational methods to analyze the structural basis for recognition. Three of the eight are Co-Principal Investigators and five are Participating Scientists (primarily for budgetary reasons). Specific problems to be addressed by the Project include: (1) How NarL and NarP, the response regulators of two-component bacterial signaling cascades, are activated for DNA binding by phosphorylation, and how they recognize and bind to their specific gene control sites. (2) How the base sequence and geometry of DNA dictate its binding to proteins and drugs, including inhibitors of the AIDS virus reverse transcriptase and side-by-side minor groove binding molecules. (3) How fully sequenced genomes can be used to identify interacting networks of macromolecules and previously uncharacterized protein assemblies. (4) How domain swapping influences protein aggregation, and whether it is involved in building the amyloid state. (5) Developing a new method for designing self-assembling proteins, and understanding how the design principles relate to biological assemblies, including cages and filaments. (6) Determination of the structure of eukaryotic glutamine synthetase as a step toward development of an anti-tuberculosis drug, and understanding of protein-ligand recognition. Although this list of projects by the three Co-PIs is diverse, the common thread through them all is that local structure controls interactions between macromolecules, and that recognition of one molecule by another is an essential step in the building of larger functional intermolecular assemblies. Space limitations prevent discussion of projects of Participating Scientists here, but their goals are similar.

Grant: 1P01GM064676-01
Program Director: NORVELL, JOHN C.
Principal Investigator: CROSS, TIMOTHY A PHD CHEMISTRY, OTHER
Title: Membrane Protein Structural Genomics: M. tuberculosis
Institution: FLORIDA STATE UNIVERSITY TALLAHASSEE, FL
Project Period: 2001/09/28-2006/08/31

DESCRIPTION (provided by applicant): Membrane proteins represent one of the greatest structural genomics challenges today. These proteins are of unquestionable importance as they exist at the interface between a cell's exterior and interior. They represent the majority of today's drug targets. And yet we have so little structural information on these proteins. Here we present a plan that will enhance the rate at which membrane protein structures will be achieved by an order of magnitude and likewise reduce the cost per structure by an order of magnitude. 13 collaborating investigators from 8 institutions bring a wealth of expertise in membrane protein production and a diversity of technologies to bear on this important problem. In bringing such a team together great opportunities exist for synergy. Knowledge will be spilled across disciplinary boundaries facilitated by a substantial effort to make communication both easy and productive. Our structural efforts will be targeted against the membrane proteins of Mycobacterium tuberculosis, the world's leading cause of infectious mortality. An effort will be made to express each of the nearly 1200 Open Reading Frames that represent membrane proteins. It is anticipated that 10 to 20 percent of these proteins will be expressed in first two years of funding. For each expressed protein, sample preparation will be attempted for at least two different technologies, such as 3D crystals for X-ray crystallography and aligned bilayers for solid state NMR spectroscopy. It is our stated aim that this team will solve 50 membrane protein structures. This would nearly triple the number of membrane protein structures in the Protein Data Bank. More importantly, it would be a major step forward in characterizing membrane protein folds and would represent a treasure trove of structural information for combating this deadly organism.

Grant: 2R01GM019756-30

Program Director: RHOADES, MARCUS M.

Principal Investigator: DAHLBERG, ALBERT E MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC

Title: Polysomes and Subunits: Structure-Function Relationship

Institution: BROWN UNIVERSITY PROVIDENCE, RI

Project Period: 1975/09/01-2005/08/31

DESCRIPTION (provided by applicant): The long-term objective of this grant proposal is to understand how the ribosome functions at the atomic level during protein synthesis. Specific goals include developing a genetic system in the extreme thermophile *Thermus thermophilus* to construct and isolate mutants in rRNA and ribosomal proteins. Mutant ribosomes which form alternate conformations in the translation cycle will be crystalized (in collaboration with Drs. Jamie Cate and Venki Ramakrishnan). A second goal is to investigate dynamic aspects of *E. coli* ribosomes by utilizing a series of genetic and biochemical methods developed in our laboratory over several years. The primary approach involves the construction of rRNA mutations in a plasmid-borne *rrn* operon. A variety of strains and vectors permit even lethal mutations to be isolated. The atomic coordinates provided by crystal studies of the 30S and 50S subunits will be used in designing mutagenic strategies to probe ribosome structure and function during peptide bond formation, translocation and decoding. Mutants forming stable functional intermediates will be characterized by a variety of functional assays and studied by cryoEM (in collaboration with Dr. Joachim Frank). Some of these mutants will be constructed and expressed in *Thermus thermophilus* for crystal studies. A third goal involves the application of these same methods to probe the mechanism of action and resistance of antibiotics affecting protein synthesis. These studies should provide fundamental insights about ribosome function and conformational changes in the rRNA and ribosomal proteins involved in tRNA selection, translocation, peptide bond formation and signal transmission within and between the subunits.

Grant: 2R01GM020478-28

Program Director: CHIN, JEAN

Principal Investigator: DOWHAN, WILLIAM PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC

Title: Structure and Function of Membrane Proteins

Institution: UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX
HOUSTON

Project Period: 1976/06/01-2005/03/31

DESCRIPTION: (Adapted from the Investigator's abstract): Several members of the Major Facilitator Supertamily (MFS) are defective in active solute transport when expressed in mutants of *Escherichia coli* lacking phosphatidylethanolamine (PE). The molecular basis for dysfunction in lactose permease (LacY) was established to be a requirement for PE as a molecular chaperone in the conformational maturation of LacY after membrane insertion. A large cytoplasmic domain in the middle of LacY when assembled in PE-lacking cells is topologically mis-assembled and exposed to the periplasm. A major aim of this proposal is to establish the molecular determinants within polytopic membrane proteins that in cooperation with membrane lipid composition and the protein assembly machinery dictate the topological organization of membrane proteins. The primary molecular probe will be accessibility of single cysteine replacements within a cysteine-lacking derivative of LacY. The topological organization of LacY and its interaction with PE will be studied using both *in vivo* and *in vitro* assembly of LacY in the presence and absence of PE. The structural and functional properties of LacY will be studied in proteoliposomes reconstituted from defined lipid components. Interaction between lipids and LacY will be studied in detergent-lipid mixed micelles. Site directed mutagenesis aimed at putative topogenic signals within LacY will be used to define elements within LacY that determine its topology. Second site suppressors of dysfunction of LacY will be isolated to identify elements of LacY and other components that determine protein topology. The phenylalanine permease and the aromatic amino acid permease are dysfunctional in PE-lacking cells. The same approaches will be used to study the molecular basis for the dysfunction of these transporters and to establish the generality of the involvement of PE in assembly of members of the MFS. The development of strains lacking PE has provided versatile biological reagents to probe the role of lipids in cell function. To broaden the scope of reagents available to study the role of lipids, *E. coli* strains will be developed that either contain or replace native lipids with lipids (monoglucosyl diacylglycerol, phosphatidylcholine, phosphatidylinositol) found in other organisms. The proposed experiments are expected to define at the molecular level the role of PE and other lipids in the assembly, organization, and function of polytopic membrane proteins.

Grant: 2R01GM020509-28A1
Program Director: ZATZ, MARION M.
Principal Investigator: ZUSMAN, DAVID R
Title: Sensory Transduction and Development in a Bacterium
Institution: UNIVERSITY OF CALIFORNIA BERKELEY BERKELEY, CA
Project Period: 1976/06/01-2005/02/28

The focus of our research project is the study of sensory and signal transduction in the bacterium *Myxococcus xanthus* as cells form multicellular aggregates and undergo fruiting body development. Directed cell movements in response to self-generated signals are important for the proper development of all multicellular organisms, including higher eukaryotes which must transduce signals to coordinate growth and differentiation during embryogenesis. 1. Analysis of components of the "frizzy" signal transduction pathway - We plan to study FrzCD, FrzE, and FrzS. FrzCD, a methylated chemotaxis protein (MCP) receptor, differs from most MCPs in that it is soluble during vegetative growth but associates with the cell membrane in a dynamic manner during early development. We plan to study its structure and unique pattern of localization. We will also study FrzE, a CheA-CheY fusion protein, and search for interacting proteins. FrzS, a protein needed for S-motility, will also be characterized. 2. Characterization of the roles of newly identified Che homologues in *M. xanthus* - We have recently discovered two new chemotaxis operons in *M. xanthus* that encode many Che homologues. We plan to analyze the roles that these new signal transduction pathways play in the complex coordination of multicellular interactions required for development. 3. Search for self-generated signaling molecules required for developmental aggregation The *frgA* and *abcA* genes, both of which confer the Frz phenotype when mutated, are most likely involved in the synthesis or transport of self generated chemotaxis signals since the defect in the mutants can be complemented extracellularly. We propose to use this complementation assay to search for and purify the putative signal substances.

Grant: 2R01GM021499-25
Program Director: RHOADES, MARCUS M.
Principal Investigator: MURGOLA, EMANUEL J PHD MOLECULAR GENETICS
Title: Translational Suppression and Codon Recognition
Institution: UNIVERSITY OF TEXAS MD ANDERSON CANCER CENTER HOUSTON, TX
Project Period: 1978/03/01-2005/03/31

DESCRIPTION: The experimental idea at the basis of this proposal is that by using both classical techniques of bacterial genetics and the manipulations of modern molecular biology, we can obtain important new ribosomal RNA (rRNA) or rRNA interactive mutants in *Escherichia coli* as translational suppressors of nonsense or missense mutations, and that those rRNA mutants will provide information about and experimental tools for the further study of the role(s) of rRNA in various functional interactions of the ribosome during translation of the genetic code. In particular, those mutants will allow us to test the hypothesis that specific nucleotides, sequences, structures, regions, or domains of one or more of the rRNAs are involved in particular aspects of specificity and accuracy in polypeptide elongation and termination. Long-range goals include answering the questions: With respect to decoding specificity in the elongation and termination stages of translation, what does rRNA do? Which nucleotides, in which of the three rRNAs, do it? Which nucleotides are involved in specific functional intramolecular interactions within a given rRNA or intermolecular interactions with another rRNA, tRNAs, or other macromolecules? The specific aims are: (1) To target specific rRNA regions, structures, and sites (a) for further characterization of existing mutants and (b) to obtain new kinds of rRNA suppressors and secondary mutations that reverse one or more phenotypes of the primary suppressor mutation, and to characterize the mutant functions and interactions *in vivo* and *in vitro*. (2) To reveal and examine *in vivo*, and ultimately *in vitro*, specific tRNA-rRNA interactions by obtaining rRNA mutations that specifically affect the action of suppressor tRNA mutants altered in different parts of a tRNA molecule. (3) To devise selections or screens for rRNA mutations that reveal dynamic aspects of translation termination (or elongation) and characterize them *in vivo* and *in vitro*. Specific segments of cloned genes will be subjected to random PCR mutagenesis and, when appropriate, specific changes will be introduced by oligonucleotide-directed, site-specific mutagenesis. Cells containing well-characterized *trpA* missense or nonsense mutations (and, in some cases, mutations in the *cat* gene), will be transformed with the mutagenized plasmids and spread on plasmid selective media, and the transformants screened for suppression of the reporter gene mutations. Some recipient cells will also contain a suppressor tRNA that is cognate or not to the reporter gene mutation. Information from these studies, which will elucidate the roles of rRNA and the ribosome in translation of the genetic code in single cells and in developmental processes, could lead to development of therapeutic procedures and reagents that interfere with pathogenic bacteria and viruses and that reverse defects in the control of cell growth. In short, our findings touch upon and suggest tools and targets for manipulation of, for example, colds, crops, cattle and cancers.

Grant: 2R01GM022172-27
Program Director: IKEDA, RICHARD A.
Principal Investigator: CANE, DAVID E PHD CHEMISTRY:ORGANIC
Title: Biosynthesis of Microbial Polyketides
Institution: BROWN UNIVERSITY PROVIDENCE, RI
Project Period: 1977/08/01-2005/07/31

Ongoing studies of the enzymology of complex polyketide natural product biosynthesis will be continued and extended, with focus on the macrolide antibiotics erythromycin (1), methymycin (2), and tylosin (3), as well as the antitumor metabolite epothilone (4). Each of these metabolites is assembled by exceptionally large, multifunctional, modular proteins known as polyketide synthases (PKSs) that are closely related to fatty acid synthases, both biochemically and genetically. In addition, epothilone synthase contains additional catalytic activities belonging to the class of non-ribosomal peptide synthetases (NRPSs). A combination of chemical, enzymological, and molecular genetic techniques this being used to elucidate the molecular basis for the programming of the complex series of reactions responsible for polyketide chain elongation. The emphasis in this work is on the elucidation of the mechanisms of multi-step, enzyme-catalyzed transformations leading to formation of biologically important metabolites. It is expected that the results of these studies will be broadly applicable not only to the understanding of polyketide and other natural product biosynthetic processes in general, but will provide fundamental insights into how catalysis and molecular recognition control both product specificity and molecular diversity in Nature. 1) Deoxyerythronolide B synthase (DEBS) is a modular PKS that catalyzes the formation of 6-deoxyerythronolide B (5), the parent aglycone of erythromycin A. Individual modules of the DEBS protein, responsible for catalysis of a single round of polyketide chain elongation and functional group modification, can be expressed in *E. coli*. These modules will be used to study the biochemical basis for the specificity and selectivity of individual catalytic domains, particularly the ketosynthase (KS) domains that mediate the key polyketide chain-building decarboxylative condensation reaction. 2) The methymycin and tylosin PKSs have intriguing similarities and differences to the well-studied DEBS system. Individual modules of the methymycin/picromycin and tylactone PKSs will be expressed in *E. coli* in order to investigate their biochemical function and substrate specificity. 3) The EpoA protein, the loading module for the epothilone hybrid PKS/NRPS, will be expressed in *E. coli* in order to study the EpoA-catalyzed conversion of malonyl-CoA to acetyl-S-EpoA, the substrate for the NRPS module EpoB.

Grant: 2R01GM022441-25A1
Program Director: CHIN, JEAN
Principal Investigator: MATTHEWS, KATHLEEN S PHD
BIOCHEMISTRY:CARBOHY
ATE
Title: ALLOSTERIC TRANSITION IN LACTOSE REPRESSOR PROTEIN
Institution: RICE UNIVERSITY HOUSTON, TX
Project Period: 1979/04/01-2004/12/31

Biological systems universally employ cascades of binding or catalytic events to transmit information. The central players in these cascades are proteins with binding sites for "input" ligands that change binding or catalysis at "output" sites. Allosteric mechanisms serve as the switch by which an input signal is converted to an output signal-most commonly through conformational changes or coupled binding/folding. This pivotal signalling process is poorly understood at the atomic level, especially for multi-domain transcription factors. We propose detailed examination of this switching pathway in the lactose repressor protein (LacI). LacI inhibits transcription of the lac metabolic enzymes by binding tightly to specific operator sites within the E. coli genome. When LacI binds inducer sugar, DNA binding is diminished and the metabolic genes are transcribed. Recent crystallographic structures for various liganded forms of LacI provide snapshots of the conformational states of LacI, but give no direct information on the molecular pathway(s) between these states. A unique opportunity exists to couple recent structural information and the vast phenotypic data on LacI mutants with detailed biochemical and biophysical characterization methods developed in our laboratory to explore allosteric signal transmission in LacI at the atomic level. Ligand binding information must flow through the structure of LacI between the widely separated inducer and DNA binding sites. Structurally, this linkage is provided by the hinge helix, which is folded only in the operator-bound form of LacI. Although the end states for the LacI allosteric change are known, the molecular mechanism of signal propagation remains unknown. This proposal is designed to elucidate the structural changes within LacI in response to DNA and inducer binding and to establish the allosteric pathway for this multi-domain transcription factor. The key hypotheses to be explored are: (1) DNA sequence influences binding and allostery through effects on hinge helix folding, (2) differences between inducer and anti-inducer ligands derive from their differential effect on hinge helix folding, and (3) specific amino acid changes can disrupt the allosteric pathway and block communication between the inducer and operator sites. To examine the local structures altered in the LacI allosteric mechanism, hydrogen exchange techniques will be added to our experimental repertoire of thermodynamic, chemical, and genetic methods. With this addition, all tools are in place to uniquely detail the allosteric structural changes of a genetic regulatory protein.

Grant: 2R01GM025326-24
Program Director: ANDERSON, RICHARD A.
Principal Investigator: KLECKNER, NANCY E PHD BIOLOGY
NEC:BIOCHEMISTRY
Title: ILLEGITIMATE RECOMBINATION BY DRUG RESISTANCE ELEMENTS
Institution: HARVARD UNIVERSITY CAMBRIDGE, MA
Project Period: 1978/07/01-2005/06/30

DESCRIPTION (provided by applicant): This application addresses chromosome behavior, organization and function in bacteria and yeast. The importance of the physico-mechanical properties of chromosomes is emphasized. I. Mechanism of Tn10 Transposition. (A) Transpososome assembly and pre- and post-assembly conformations will be investigated in vitro by analytical and crosslinking methods. (B) Structural analysis of transposase DNA complexes will be attempted, in collaboration with Dr. G. Van Duyne. II. E.coli DNA replication, cell division and chromosome organization. (A) Synchronous cell populations generated by a new baby cell method will be analyzed, by FACS, FISH, immunocytology and DNA assays, in wild type and mutant conditions. Roles of newly identified negative regulators and basic features of a new general model will be assessed. (B) Chromosome organization and physical properties will be analyzed by our new crosslinking method. (C) cis and trans coupling between/among different chromosomes and chromosomal regions will be probed by new methods. III. Eukaryotic chromosome organization and function. We will investigate basic processes in the context of chromosome breathing and concomitant chromosomal stress and stress relief. (A) Chromosome status will be analyzed using our newly developed crosslinking assay in wild type and genetically altered situations. Issues of interest include: R-band/G-band differences, chromosome expansion and relaxation, origin status and intersister relationships. (B, C) Coordinated studies in *Sordaria* and yeast will investigate Spo76/Pds5, proposed to be a transducer of expansional stress, and bulk chromatin proteins that could directly modulate chromatin/chromosome expansion (Bdf1/2, H1, HMG6AB and histone H3). (D) Studies of yeast DNA replication and cell cycle progression will investigate the role of newly discovered inter-origin elements (IOEs) in regulation of S-phase progression via Mec1. We will begin to investigate the hypothesis that cell cycle progression is governed by SLS's (sensors of local stress) and that Mec1/Esr1 is an integral component of this machinery and is a stress-dependent kinase.

Grant: 2R01GM025349-24
Program Director: PREUSCH, PETER C.
Principal Investigator: SENIOR, ALAN E PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: CHARACTERIZATION OF E.COLI F1FO-ATP SYNTHASE
Institution: UNIVERSITY OF ROCHESTER ROCHESTER, NY
Project Period: 1978/09/01-2005/08/31

Synthesis of ATP during oxidative phosphorylation occurs on the F1Fo-ATP synthase enzyme and accounts of the bulk of ATP synthesis in living cells. ATP synthase is an extraordinary enzyme because it acts as a molecular motor the energy of the transmembrane proton gradient is coupled through subunit rotation to the synthesis of ATP in three asymmetric but interconverting catalytic sites. In reverse, ATP hydrolysis drives subunit rotation and proton pumping. The long-term goal of this research is to understand the mechanism of F1Fo-ATP synthase in as much molecular detail as possible. The E. coli enzyme will be used because of its many advantages, e.g. it is readily amenable to molecular biology/genetic manipulations, it may be rapidly obtained in high yield, and it may be reconstituted in liposomes with excellent ATP synthesis activity. Specific goals are (1) determination of catalytic sites occupancy and nucleotide binding parameters in F1Fo in presence of a proton gradient, during ATP synthesis; (2) identification of functional interactions between gamma/alpha and gamma/beta subunits, by mutagenesis of residues in gamma which face alpha and beta; (3) elucidation of protein movements generated at the catalytic alpha/beta subunit interface as ATP hydrolysis proceeds through formation and collapse of the transition state to the ADP ground- state; (4) characterization of ATP hydrolysis at low ATP concentrations, where 120 degrees Celsius stepping of the rotor is seen; and (5) genetic analysis of the stator stalk, starting from mutations in alpha and delta subunits shown previously in this laboratory to interrupt both F1 binding to Fo and energy coupling. ATP-driven pumps are very widely distributed in nature, and are involved in many disease states. Work to be done here will consequently have broad impact in biology and medicine.

Grant: 2R01GM028216-25A2
Program Director: ANDERSON, JAMES J.
Principal Investigator: MICHELS, CORINNE A
Title: Maltose sensing/signaling mechanisms in *Saccharomyces*
Institution: QUEENS COLLEGE FLUSHING, NY
Project Period: 1980/07/01-2005/06/30

The goal of this project is to characterize two maltose sensing/signaling pathways identified in *Saccharomyces*. The first regulates inducible MAL gene transcription via maltose permease and the MAL-activator. The second stimulates enhanced levels of pseudohyphal differentiation and is distinct from the pathway regulating MAL gene expression. Specific Aim 1 investigates the role of maltose permease in regulating MAL gene expression. Is it a maltose receptor? Methods are described to isolate constitutive mutations in maltose permease and in other proteins that might function as downstream components of the postulated maltose signaling pathway. Additionally, we will test the ability of a heterologous maltose transporter to induce MAL gene expression. Specific Aim 2 investigates the intracellular sorting of maltose permease during synthesis. Included is an analysis of glucose-induced changes in this trafficking pattern and the role of Snf1 kinase. Snf1 kinase is required posttranscriptionally for maltose permease synthesis. Suppressor analysis and biochemical approaches are described to identify this function. The roles of Std1p and casein kinase I (Yck1,2p) in the glucose-induced proteolysis of maltose permease will be investigated. Specific Aim 3 explores the role of Hsp90 molecular chaperone in the maltose-induced activation of the MAL-activator. The MAL-activator is an Hsp90 chaperone client protein. We will use our collection of MAL-activator mutants to test a proposed chaperone-mediated model of induction. Mutant MAL-activator turnover, chaperone complex formation, and response to permease overexpression will be assayed. Carbon source regulation of MAL-activator degradation will be examined and the ubiquitin-conjugation pathway components determined, particularly the E3 ubiquitin-protein ligase. Specific Aim 4 will define the upstream components of the maltose sensing/signaling pathway used to stimulate pseudohyphal differentiation, including the maltose receptor. Gpr1 receptor and Gpa2 Galpha protein are not utilized. Tpk2 kinase (PKA) is required but no maltose-stimulated increase in cAMP synthesis is seen. Mutants capable of filamentation on glucose but not maltose will be isolated using transposon-mutagenesis.

Grant: 2R01GM028485-21

Program Director: SOMERS, SCOTT D.

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

Title: MOLECULAR PATHOLOGY OF LPS INDUCED SHOCK AND DIC

Institution: SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA

Project Period: 1981/03/01-2005/06/30

Sepsis may result in to 250,000 deaths annually in the United States. The development of septic shock in sepsis accounts for many of these deaths. Septic shock results from dysregulation of the innate immune response to infection. Past studies have focused on Gram- negative (G-) bacteria emphasizing the role of bacterial endotoxin (LPS) in the pathogenesis of the disease. Basic and clinical studies with LPS have revealed many fundamental mechanisms of the innate immune system. In contrast few studies mechanistic studies have been performed with Gram-positive (G+) organisms. This has primarily occurred because of doubts about the importance of G+ bacteria in septic shock. Now leaders in the field recognize that G+ infection is likely to be an important cause of septic shock. A currently held view is that there are overlapping an distinct immune/inflammatory responses to G- and G+ organisms. Unfortunately few efforts have been made to dissect the molecular mechanisms of the innate immune response to G+ bacteria. Here we describe experiments to bridge this significant gap in out knowledge. To do this we propose a series of studies to define the structure and function of Toll-like receptor 2 (TLR2), the receptor for G+ bacteria. We will use quantitative biochemical and molecular biological approaches in studies to address the four Specific Aims proposed herein. Our Aims speak to our major long-term goal; to fully define the composition of TLR2 receptor complex and to identify key steps in the earliest signaling events that ultimately control gene expression. In addition we will also use mice containing gene deletions of key TLR family members in the cecal ligation model of bacteremia. The goal of the latter studies is to better understand the interrelationships among the various TLRs in the setting of bacteremia. The data provided by our studies will lead to a new understanding of the pathophysiological responses to sepsis through an understanding of the basic mechanisms of the innate immune response. By doing this we hope to identify new therapeutic targets and to highlight proteins to consider in future studies investigating the genetic basis of septic shock.

Grant:	2R01GM030367-20	
Program Director:	SCHWAB, JOHN M.	
Principal Investigator:	WHITESIDES, GEORGE M	PHD CHEMISTRY:CHEMISTRY- UNSPEC
Title:	Multivalency: Mechanisms and Applications	
Institution:	HARVARD UNIVERSITY	CAMBRIDGE, MA
Project Period:	1982/04/01-2005/06/30	

This research will develop multivalency as a strategy for modulating biological interactions. Multivalent interactions are those that involve multiple receptors interacting simultaneously with multiple ligands. Multivalent interactions are ubiquitous in biology-in infectious disease, in processes involving antibodies, in blood clotting, metathesis, platelet activation, and inflammation, and in many conditions in which cells interact with surfaces-but are seldom explicitly the target of study or of therapy. Drugs are designed primarily on the principle of "one drug, one receptor". The objective of this research is to explore a range of multivalent interactions using multivalent ligands as probes, to understand those types of biologically relevant interactions that are most easily controlled by synthetic, multivalent entities, and to demonstrate proof-of-concept in the use of multivalent species as drugs. Test systems will be drawn primarily from infectious disease: E. coli, pseudomonas, B. anthracis, and influenza are of particular interest. It will also develop new analytical systems that are tailored to the special requirements of multivalency.

Grant: 2R01GM035269-16A1
Program Director: DEATHERAGE, JAMES F.
Principal Investigator: EGELMAN, EDWARD H PHD
BIOPHYSICS:MOLECULAR
STRUCTURE
Title: Structural Studies of Nucleoprotein Complexes
Institution: UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA
CHARLOTTESVILLE
Project Period: 1986/04/01-2005/02/28

DESCRIPTION (provided by applicant): Much of the research on homologous recombination has been focused on the bacterial RecA protein. The eukaryotic Rad51 protein forms very similar nucleoprotein filaments to those formed by RecA. Rad51 is essential in higher organisms, including humans, and is believed to play a large role in the maintenance of genome stability. Electron microscopic (EM) studies of these protein-DNA filaments can yield important information about mechanism in these highly conserved structures. Significant improvements in resolution are now possible using cryo-EM of frozen-hydrated specimens, as well as new computational approaches. These studies are beginning to show how domains are arranged in these filaments, the conformational changes that are associated with activation of the filaments, as well as revealing interactions between these filaments and other proteins. Helicases have the same nucleotide-binding core present in the RecA and Rad51 proteins. It has become apparent that a large number of helicases involved in DNA recombination, replication, repair and transcription form hexameric rings around DNA. Mutations in helicase genes have been shown in humans to lead to xeroderma pigmentosa, Cockayne's syndrome, Werner's syndrome and Bloom's syndrome. There are numerous suggestions that many of these proteins may not actually be helicases, but motor proteins with diverse functions. In fact, more than 2 percent of human genes may encode for "helicases." EM and single particle image analysis will be used on different hexameric replicative helicases, including E. coli DnaB, Simian Virus 40 large T, archaeal MCM and bacteriophage T7 gp4, to study the interaction with DNA, conformational changes that occur during the ATPase cycle, and cooperative interactions among subunits. These studies will be done in collaboration with crystallographic efforts, and high-resolution data on subunits and domains will be combined with lower resolution data on quaternary organization. The overall question that will be addressed is whether the conserved nucleotide-binding core present in RecA, Rad51 and the helicase superfamily has led to a conservation of mechanism across this huge class of proteins. Structural studies are now revealing that other proteins active in recombination and repair, with no apparent homology to this class, also form rings. Examples are translin, involved in chromosomal translocation and RNA processing, and Rad52. Studies of these proteins will be conducted in parallel, with the aim of understanding whether convergent mechanisms of protein-mediated recombination and repair exist.

Grant: 2R01GM035769-15A1
Program Director: RHOADES, MARCUS M.
Principal Investigator: BELASCO, JOEL G PHD
Title: Prokaryotic RNA Metabolism
Institution: NEW YORK UNIVERSITY SCHOOL OF NEW YORK, NY
MEDICINE
Project Period: 1986/01/01-2005/03/31

DESCRIPTION: These investigations will focus on elucidating the process of mRNA degradation in *Escherichia coli*. The goal of these studies will be to identify and characterize RNA elements responsible for differences in mRNA stability, to define the features of a key bacterial ribonuclease that determine its specificity and function, and to elucidate important aspects of the mechanism by which this ribonuclease acts. Molecular biological, biochemical, and genetic methods will be employed. Particular attention will be devoted to RNA degradation by RNase E, a ribonuclease thought to control the principal pathway for mRNA decay in *E. coli*. First, we will examine how the rate of internal RNA cleavage by this endonuclease is controlled by the phosphorylation state of the RNA 5' terminus. In addition, we will investigate the RNA and protein elements that govern the ability of RNase E to autoregulate its synthesis in *E. coli*, taking advantage of a powerful and convenient genetic system that we have developed for this purpose. Finally, we will examine the role of 5'-terminal RNA secondary structure as an impediment to mRNA degradation by the related *E. coli* endonuclease CafA. The results of these studies should enhance our knowledge of a fundamental aspect of gene regulation that presently is poorly understood. This knowledge should ultimately be of value in maximizing bacterial production of medically useful proteins and in clarifying a biological regulatory mechanism that can play an important role in microbial pathogenesis.

Grant: 2R01GM036936-14A1
Program Director: DEATHERAGE, JAMES F.
Principal Investigator: KHAN, SHAHID M PHD
Title: Motility and Chemosensing in Bacteria
Institution: UPSTATE MEDICAL UNIVERSITY BRONX, NY
Project Period: 1986/07/01-2005/03/31

DESCRIPTION: (Adapted from the Investigator's abstract): The overall objective is determination of the assembly and operation of the bacterial flagellar rotary motor. The research will enhance understanding of the molecular basis of motility and its regulation, chemiosmotic energy transduction and conformational switching in macromolecular assemblies. These fundamental issues are key to deciphering how living cells work. The understanding gained will be relevant, most immediately, for control of bacterial pathogens, and, given time, for cell based diagnoses of metabolism-related diseases. The rotor component of the *Salmonella typhimurium* flagellar motor has been over-produced in abundance by ho-expression of the four proteins (FliF, FliG, FliM, FliN). Scanning transmission electron microscopy (STEM) will aim to relate protein copies to subunit number through analysis of mass differences between complete and partial rotors containing a limited set of components. Cryoelectron microscopy (Cryo-EM) and metal replication will resolve subunit architecture and its change in mutant rotors locked in clockwise (CW) or counter-clockwise (CCW) rotating configurations. The number and location of CheY binding sites, will be determined utilizing high-density-gold labeled CheY, and related to associated shifts in subunit tilt. More comprehensive 3D-reconstruction maps, in addition to analysis of the switching mechanism, should provide a basis for identification of the flagellar protein export apparatus and its interaction with the motor machinery. Strains co-expressing the stator MotA, MotB proteins with the rotors have impaired growth, implying reconstitution of proton transport. Together with proton flux measurements, formation of intra-membrane particle rings will be monitored to define the role of FliG and FliM protein domains in docking, proton transport and, eventually, rotation. Mutation-induced changes in CheY-rotor affinity will be measured by evanescent wave fluorescence microscopy. These measurements will be made both in immobilized cells or envelopes, utilizing green fluorescent protein (GFP) or rhodamine-tagged CheY proteins respectively. They should provide novel information on the energetic basis for the high co-operativity of the flagellar motor switch, complementary to the planned structural studies.

Grant: 2R01GM038130-09
Program Director: PREUSCH, PETER C.
Principal Investigator: BUTLER, ALISON
Title: THE BIOINORGANIC CHEMISTRY OF IRON
Institution: UNIVERSITY OF CALIFORNIA SANTA BARBARA, CA
BARBARA
Project Period: 1988/02/01-2005/01/31

DESCRIPTION (Adapted from applicant's abstract): The objectives of this research are to improve our understanding of the mechanisms of metal acquisition by microorganisms in the marine environment. Interest in the mechanisms of acquisition of iron (and other metal ions) by oceanic bacteria derives from the unique transition metal ion composition of the ocean and the discrepancy between iron availability and requirements. Iron is a limiting nutrient to marine microorganisms over much of the world's oceans at a concentration of 0.02-1 nM in surface seawater, whereas molybdenum and vanadium are the two most abundant transition metal ions at 100 nM and 20-35 nM, respectively. In the previous grant period the Principal Investigator discovered a new class of self-assembling amphiphilic peptide siderophores, the marinobactins and aquachelins, produced by two phylogenetically distinct genera within the marine gamma proteobacteria. The only siderophores which bear a structural resemblance to the marinobactins and aquachelins are the amphiphilic mycobactin and exochelin siderophores. These siderophores are produced by mycobacteria, such as *Mycobacterium tuberculosis*, the bacterium causing tuberculosis. Little is known about the molecular mechanism of pathogenesis of *M. tuberculosis*, however, its capacity to infect the host is closely linked to its ability to acquire iron. The specific aims of the proposed research include I) further characterization of the amphiphilic marine siderophores and the mycobactins, II) further investigations of the *Alteromonas luteoviolacea* system, the marine bacterium which produces the alterobactin siderophores and III) the isolation and structural characterization of siderophores produced by other oceanic bacteria. These studies are the first part of an investigation into whether the mechanisms of iron acquisition (e.g., siderophore-mediated sequestration of the iron, outer membrane receptor protein recognition of the metal siderophore complex, transport, and metal regulation of these processes, etc.) by marine bacteria differ from terrestrial bacteria.

Grant: 2R01GM038237-15
Program Director: PREUSCH, PETER C.
Principal Investigator: DALDAL, M F
Title: MOLECULAR GENETICS OF THE CYTOCHROME BC1 COMPLEX
Institution: UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA
Project Period: 1987/12/01-2005/01/31

A vital characteristic of living systems is their ability to perform efficient energy conversion. Biological energy transduction is the ensemble of pathways necessary for cellular energy (ATP) production, which is essential for many cellular functions, including macromolecular biosynthesis, solute transport, signal transduction, chemotaxis, phototaxis and thermogenesis. The long term goal of this project is to define the cytochrome (cyt) components of these pathways, and to understand their structure, mechanism of function and biogenesis (i.e., assembly, maturation and regulation). Facultative phototrophic bacteria (e.g., *Rhodobacter* species) provide an excellent model system for eukaryotic organelles. The energy transduction complexes are widespread among living organisms, and their improper function leads to devastating neuromuscular and mitochondrial diseases in humans, and low crop yields in plants. This project will continue molecular and biochemical genetics of these complexes, focusing on the structure, function and biogenesis of membrane-bound, multisubunit cyt c complexes (i.e., the ubiquinone: cyt c oxidoreductase, or the bc1 complex, and the cbb3-type cyt c oxidase). Major progress has recently been accomplished by the resolution of the 3D structure of the bc1 complex, and recognition of the mobility of its FeS protein subunit. In the light of these excitements, the specific aims include the analysis of the FeS protein motion and its control during Q0 site catalysis; direct and rapid activation of the bc1 complex using photoactivatable compounds to study the movement of the FeS protein as a unique intra-protein electron shuttle device; construction of novel cyt c1 molecules with unusual ligand and folding properties; engineering of novel bc1 complex variants to correlate their structural flexibility and functional similarities; and genetic and biochemical studies of mutants and gene products involved in the biogenesis of multisubunit cyt c complexes and incorporation of their prosthetic group. These studies will increase significantly our understanding of the structure and mechanism of function of the bc1 complex as a prototype for a unique intra-protein electron shuttle device, and pave the road to future "single molecule" studies. They will also provide important insights into the biogenesis of membrane-bound multisubunit cyt c complexes that operate during cellular energy production, an important biological process that is far from being completely understood, even in bacteria. Finally, insights gained in this simpler system are generally applicable to the structurally more complex and yet functionally similar organelle-derived complexes, and are important for the elucidation of the molecular basis of mitochondrial diseases and aging.

Grant: 2R01GM038681-14A1

Program Director: CHIN, JEAN

Principal Investigator: KADNER, ROBERT J

PHD

BIOCHEMISTRY:BIOCHEM

RY-UNSPEC

Title: EXOGENOUS INDUCTION OF THE E.COLI UHPT GENE

Institution: UNIVERSITY OF VIRGINIA

CHARLOTTESVILLE, VA

CHARLOTTESVILLE

Project Period: 1987/07/01-2004/11/30

DESCRIPTION (Adapted from applicant's abstract): This project seeks to elucidate how an extracellular signal changes specific gene transcription. The *Escherichia coli* uhp locus encodes an active transport system, UhpT, for uptake of sugar phosphates and related substrates. Transcription of the uhpT gene is induced by extracellular glucose-6-phosphate through the action of an unusual two-component regulatory system encoded by the uhpABC genes. Our overall goal is to understand the mechanisms of transmembrane signaling by the UhpBC genes. Complex, the regulation of phosphate transfer to the transcription activator UhpA, the effect of phosphorylation of UhpA on its function, the mechanism of transcription activation by UhpA, and the basis for the co-dependent activation of transcription by UhpA and the global regulatory protein CAP. 1. The segments of UhpB that are involved in recognition of UhpA, and phosphate transfer to and from it, will be probed by genetic and biochemical tests. The role of sequestration of UhpA on the proper control of gene expression will be analyzed. The basis for the inactivity of the isolated kinase segment as auto-kinase will be explored as a function of the exposure of the site of phosphorylation, and the effect of mutations and the presence of UhpC on conformation of the phosphorylation domain. 2. The interactions of the membrane-bound UhpB and UhpC proteins in response to the presence of inducer will be examined by chemical crosslinking studies. The reconstitution of purified full-length UhpB into proteoliposomes, with and without UhpC, will allow biochemical examination of the inducer-specific control of phosphate transfer reactions by UhpB. The role of the hydrophobic N-terminal half of UhpB in membrane anchorage, binding of UhpC, and signal transduction process, by deletion or replacement of individual transmembrane segments. 3. The UhpC protein will be purified and reconstituted into proteoliposomes for assay of inducer binding and transport. Genetic screens will analyze the role of segments of UhpC in inducer binding and specificity. Chimeric proteins containing portions of UhpC and of the transporter UhpT will be made to identify the protein segments that differentiate between the signaling activity of UhpC and the transport activity of UhpT. These studies should provide new information on transmembrane signal transduction coupled between two membrane proteins.

Grant: 2R01GM040388-12
Program Director: PREUSCH, PETER C.
Principal Investigator: KURTZ, DONALD M
Title: Bacterial Proteins Containing Novel Iron Sites
Institution: UNIVERSITY OF GEORGIA ATHENS, GA
Project Period: 1988/07/01-2005/03/31

DESCRIPTION: (provided by applicant) The overall goal of this research is to determine the roles of two types of diiron-oxo proteins, ruberythrin (Rbr) and hemerythrin (Hr) homologs in the oxidative stress response of the anaerobic, sulfate-reducing bacterium, *Desulfovibrio vulgaris*. A comprehensive set of in vitro and in vivo experiments is proposed in order to provide the framework for understanding the roles of these proteins in the oxidative stress response. A detailed molecular characterization, including an X-ray crystal structure, of the reactive form of Rbr is proposed. Hydrogen peroxide is implicated as the substrate for Rbr. Therefore, measurements of the kinetics of oxidation of Rbr by hydrogen peroxide using stopped-flow spectrophotometry are proposed. Characterization of the ability of Rbr to function as the terminal component of a peroxidase upon combination with one or more of the ancillary proteins is also proposed. The gene for a proposed regulator of the oxidative stress response, Fur, will be probed by deletion of the gene encoding Fur in the *D. vulgaris* chromosome and identification of the proteins whose expression is altered in this Fur-deletion strain. Hrs have heretofore been found only in marine invertebrates, in which it functions as an oxygen uptake and storage protein. We recently discovered Hr homologs in *D. vulgaris*. One of these Hr homologs forms a domain of a larger protein whose role is to sense small molecules in its environment and to initiate a chemotactic response in *D. vulgaris*. We propose that this chemotaxis protein, called DcrH, senses oxygen, and causes the bacteria to swim towards lower oxygen concentrations. This anaerotactic response will be tested in a dcrH deletion strain. DcrH is the first characterized Hr homolog in any microorganism. An increasing number of Rbr and Hr homologs are being discovered in infectious anaerobic microorganisms. Therefore, these proposed studies would form the basis for understanding oxidative stress not only in *D. vulgaris*, but also in a diverse range of anaerobic microorganisms, several of which affect human health.

Grant: 2R01GM040731-14
Program Director: FLICKER, PAULA F.
Principal Investigator: FALKE, JOSEPH J PHD
Title: Activation and Dynamics of Receptors and Kinases
Institution: UNIVERSITY OF COLORADO AT BOULDER BOULDER, CO
Project Period: 1988/07/01-2005/06/30

The continuing project investigates the broad class of two-component signaling pathways, recently found to be widespread eukaryotes and ubiquitous in prokaryotes. Two-component pathways play especially critical roles in bacteria, where they control diverse cellular functions including cell division, antibiotic resistance, activation of virulence, and wound detection during infection. The receptors and signaling proteins which comprise these ancient pathways are conserved across species and are considered to be attractive targets for broad-spectrum antibiotics. Thus, a basic mechanistic understanding of the pathways components will have significant impacts on signaling biology and pharmaceutical development. The present research program focuses on two central elements of the chemotaxis pathway of *Escherichia coli* and *Salmonella typhimurium*: the transmembrane aspartate receptor and the cytoplasmic CheA kinase it regulates. The goals address four fundamental questions. First, how does attractant binding to a chemoreceptor generate a transmembrane signal? Second, how does a receptor adapt to a constant background stimulus? Third, how do these distinct attractant and adaptations signals modulate the activity of a receptor-bound kinase? Fourth, how do the components of the pathway assemble into a cooperative, multi-protein signaling complex? Novel approaches utilizing site-directed cysteine chemistry and spectroscopy are being utilized to address these questions. The progress reports describes the chemical determination of a low-resolution structure for the cytoplasmic domain of the aspartate receptor, and the identification of regions of the cytoplasmic domain critical for CheA kinase regulation. In addition, a highly dynamic region of the cytoplasmic domain is described, and working models for the mechanism of receptor-regulated kinase regulation are proposed. The specific aims utilize site-directed cysteine chemistry, fluorescence and EPR spectroscopy to further investigate the mechanism by which signals are transmitted through the receptor to the kinase, and the effects of these signals on kinase structure, dynamics and activity. The geometry of the assembled receptor-kinase signaling complex is also probed. Overall, the broad goal of these studies is to understand the mechanisms of transmembrane signaling, receptor adaptation, and kinase regulation in a fully assembled, multi-protein signaling complex.

Grant: 2R01GM041376-13
Program Director: CHIN, JEAN
Principal Investigator: EBRIGHT, RICHARD H
Title: BACTERIAL TRANSCRIPTION COMPLEXES
Institution: RUTGERS THE ST UNIV OF NJ NEW BRUNSWICK, NJ
BRUNSWICK
Project Period: 1988/12/01-2004/11/30

Transcription initiation by bacterial RNA polymerase (RNAP) involves a series of steps: (i) RNAP binds to promoter DNA, yielding an RNAP-promoter closed complex; (ii) RNAP clamps tightly onto promoter DNA, yielding an RNAP-promoter intermediate complex; (iii) RNAP melts equal to approximately 14 nucleotides of promoter DNA, yielding a catalytically competent RNAP-promoter open complex; (iv) RNAP initiates synthesis of RNA, yielding an RNAP-promoter initial transcribing complex; and (v) RNAP breaks its interactions with promoter DNA--"escapes"--yielding an RNAP-DNA elongation complex. Each of these steps is a potential target for transcriptional regulators. Understanding transcription initiation and transcriptional regulation will require defining the structure of the RNAP-promoter complex at each step defining the structural transitions at each step, defining kinetics of structural transitions, and defining mechanisms by which regulators affect structural transitions. The proposed work will use fluorescence resonance energy transfer (FRET), single-molecule FRET, stopped-flow FRET, and kinetic photocrosslinking to address four specific aims: Specific Aim 1: To analyze the structure of RNAP holoenzyme. Specific Aim 2: To analyze the structures of trapped RNAP-promoter complexes. Specific Aim 3: To analyze the mechanism of entry of RNAP into promoter DNA. Specific Aim 4: To analyze the mechanism of escape of RNAP from promoter DNA. The results will contribute to understanding bacterial transcription initiation, to understanding bacterial transcriptional regulation, and to design and synthesis of low-molecular-weight inhibitors of bacterial transcription, for application in antimicrobial therapy. Since eukaryotic RNAP subunits show sequence, structural, and mechanistic similarities to bacterial RNAP subunits, the results also will contribute to understanding eukaryotic transcription initiation and regulation.

Grant: 2R01GM041560-19
Program Director: SCHWAB, JOHN M.
Principal Investigator: WILLIAMS, DAVID R
Title: Chemical Synthesis of Novel Natural Products
Institution: INDIANA UNIVERSITY BLOOMINGTON BLOOMINGTON, IN
Project Period: 1982/04/01-2005/06/30

DESCRIPTION: (provided by applicant) - This research program is broadly directed to investigate fundamental advances for the chemical synthesis of biologically active marine natural products. Part I. Marine Antitumor Macrolides. Section A. A plan for the highly efficient, convergent, and enantiocontrolled synthesis of laulimalide will be executed. Laulimalide displays comparable potency to taxol with IC₅₀ concentrations in the 0.01-0.05 μ M range against a broad selection of tumor cell lines. High cytotoxicity was registered toward KB lines (IC₅₀ = 0.015 μ M). Significantly laulimalide retains activity in multi-drug resistant SKVLB-1 cultures. Section B. Strategies for the synthesis of peloruside will explore issues of stereoselectivity and efficiency for the assembly of this highly oxygenated hemiketal. With key structural features and macrocyclic rigidity, which address important proposals and research concerning the nature of the antitumor activity of the bryostatins (NCI; phase II trials), peloruside A studies are designed to offer a significant chemical advance for probing the nature of the pharmacophore and the mechanisms of biological activity for this family of molecules. Our chemical studies toward these target molecules will develop asymmetric allylation reactions. Bidirectional, divergent asymmetric allylation strategies are examined. Direct formation of new homochiral allylborane species will be pursued via cross coupling reactions with organozinc reagents. Part II. Zoanthamines. Our investigations of this novel class of marine alkaloids describe challenging issues of chemical synthesis toward densely functionalized, polycyclic systems. Members of this class have exhibited important antitumor and anti-inflammatory activities, and norzoanthamine is considered to be a promising osteoporotic candidate. Part III. Australifungin. This unique natural product is a potent antifungal which is the first nonsphingosine-based inhibitor of sphingolipid biosynthesis. It functions as a selective inhibitor of sphinganine N-acyl transferase, and may have an important role in lipid signal transduction, cell differentiation, and apoptosis. Our plans toward zoanthamines and australifungin will explore asymmetric induction in conjugate addition reactions and intramolecular Michael-based cyclizations. The intramolecular (4+2) cycloadditions of nitroalkene precursors will provide facile construction of trans-decalins. .

Grant: 2R01GM042077-17
Program Director: ANDERSON, JAMES J.
Principal Investigator: PRICE, CHESTER W PHD
Title: Molecular Genetics of Bacillus subtilis RNA Polymerase
Institution: UNIVERSITY OF CALIFORNIA DAVIS DAVIS, CA
Project Period: 1988/08/01-2005/07/31

DESCRIPTION (provided by applicant): Exposure of bacteria to diverse growth-limiting stresses induces the synthesis of a common set of proteins that provides broad protection against future lethal challenges. This general stress response enhances survival in the natural environment, in fresh and processed foods, and in certain pathogenic interactions. Among *Bacillus subtilis* and related Gram positive pathogens, this response is governed by the sigmaB transcription factor. Loss of sigmaB function causes increased sensitivity to multiple stresses, including acid, heat, osmotic, and oxidative stress. Our long term objective is to understand this response using *Bacillus subtilis* as a model, beginning with the sensors that detect the different stresses, extending through the signal transduction network that conveys this information to sigmaB, and ending with the physiological role of the 200 or more genes under sigmaB control. Of these areas, most is known about the signal transduction network, which functions by a 'partner switching' mechanism in which formation of alternate protein complexes is controlled by serine and threonine phosphorylation. This mechanism appears to be very ancient, very plastic, and widespread among the eubacteria. Here it activates sigmaB in response to two classes of stresses: (i) energy stress, including starvation for carbon, phosphate, or oxygen; and (ii) environmental stress, including acid, ethanol, heat, or salt stress. These two classes are conveyed to sigmaB by independent upstream signaling pathways, each terminating with a differentially regulated PP2C phosphatase and converging on the two direct regulators of sigmaB, the RsbV anti-anti-sigmaB and the RsbW anti-sigmaB factor. The energy branch consists of the RsbP phosphatase (with a PAS domain important for signaling) and RsbQ, a protein of unknown function required for signaling. The environmental branch has at least nine regulators, all joining to activate the RsbU phosphatase. How energy or environmental signals enter their respective branches is unknown. Also poorly understood is how the genes in the sigmaB regulon contribute to stress resistance. Experiments using DNA arrays indicate that sigmaB controls only a few genes with a direct protective function and instead governs changes in metabolism and envelope function which may confer a passive resistance. Our three specific aims address the following questions: (1) How do energy stress signals activate their branch of the network; (2) How do environmental signals activate their branch; and (3) What are the physiological roles of newly identified members of the sigmaB regulon, particularly those that may be involved in downstream signaling?

Grant: 2R01GM044918-10
Program Director: SOMERS, SCOTT D.
Principal Investigator: REMICK, DANIEL G
Title: ROLE OF CYTOKINES IN SEPSIS AND TRAUMA
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 1996/09/30-2004/11/30

Sepsis remains a serious clinical problem causing thousands of deaths each year. Therapy for the treatment of sepsis has not progressed due to a lack of understanding of the basic inflammatory processes. The previous prevailing hypothesis postulated that patients with sepsis had too much inflammation, therefore blocking the inflammatory mediators would decrease organ injury and death. As information was gathered it became clear that this hypothesis represented an oversimplification of the complex inflammatory events. This application will study 2 hypotheses using the clinically relevant murine model of sepsis induced by cecal ligation and puncture (CLP). The first hypothesis states that death during the first 5 days after CLP is due to an exaggerated immune response and will be tested by 3 specific aims. The first specific aim will block inflammatory mediators to improve survival. This represents more than just an extension of the previous failed clinical trials since we will attempt combination immunotherapy and inhibit newly described cytokines such as interleukin 18. The second specific aim will tailor the anti-inflammatory therapy based on the individual response. Rather than providing exactly the same therapy to each animal, the therapy will be directed by rapidly quantitating the level of inflammation and then providing the appropriate therapy. The third specific aim will attempt to improve outcome by increasing the local neutrophil recruitment to the site of inflammation, the peritoneum. While neutrophils may cause organ injury, they are also a critical component of the innate immune response for the control of the invading bacteria. Our second hypothesis states that deaths which occur beyond 5 days post CLP are the result of the immunosuppressed state of the animal. The fourth specific aim will test this hypothesis by providing exogenous cytokines (tumor necrosis factor, IL-1, IL-18) to the animals and attempt to boost their immune response. These exogenous cytokines will be given by subcutaneous injection. Alternatively, inducible gene therapy will also be given using the novel tetracycline inducible vectors. For each of the specific aims we will not only test if they are effective but also determine the mechanism of how they improve survival. Successful completion of our studies will provide the basic knowledge about the inflammatory response in sepsis, knowledge needed to reduce the impact of sepsis.

Grant: 2R01GM046511-11
Program Director: LEWIS, CATHERINE D.
Principal Investigator: BECKETT, DOROTHY PHD
Title: Quantitative Studies of the Biotin Regulatory System
Institution: UNIVERSITY OF MARYLAND COLLEGE PK COLLEGE PARK, MD
CAMPUS
Project Period: 1992/05/01-2005/04/30

DESCRIPTION (provided by applicant): Protein-protein interactions are critical to regulation of expression of genetic information. The strength of homologous protein-protein interactions dictates the level of occupancy of transcriptional regulatory sites on DNA. Patterns of heterologous protein-protein interactions determine the selection of DNA target site and/or the level of occupancy of a site. Through their influence on DNA binding these macromolecular interactions are fundamental to control of expression of genetic information. Elucidation of the details necessary for successful clinical intervention at the level of protein-protein interactions in control of gene expression requires combined solution physico-chemical studies of function as well as high-resolution structural information. The *Escherichia coli* biotin regulatory system provides an excellent model system for detailed studies of the control of a genetic regulatory switch via protein-protein interactions. In this proposal experiments designed to determine the structural and thermodynamic features of corepressor-induced assembly of a transcriptional repressor are described. Strategies to examine the control of gene expression via heterologous protein-protein interactions are also outlined. Methods utilized in the proposed work include site-directed and random mutagenesis, stopped-flow fluorescence kinetic measurements, sedimentation equilibrium, DNaseI footprinting, isothermal titration calorimetry and x-ray crystallography. Results of these combined studies will yield information of general significance to understanding the detailed chemistry of the protein-protein interactions essential to regulation of genetic expression.

Grant: 2R01GM047369-08
Program Director: JONES, WARREN
Principal Investigator: LYNN, DAVID G
Title: CHEMICAL & GENETIC APPROACHES TO DEFINE CELL ACTIVATION
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 1992/04/01-2001/12/31

Sophisticated strategies have emerged to regulated the critical vegetative/parasitic transition in all pathogens, and in many cases, this regulation is provided by two-component regulatory system. The VirA/VirG system of the plant pathogen *Agrobacterium tumefaciens* is the model of a critical integrator of several different external signal inputs necessary for the commitment to pathogenesis. It is the only system for which the input signal is known and a model for signal perception exists. Moreover, the genetic and molecular tools to manipulate the genome are in place, allowing the factors that control vir gene expression to be easily and safely controlled in the pathogenetic organism itself. To seize this opportunity, we propose an experimental plan to allows us to test a molecular model for signal perception, integration, and transmission in pathogenetic signaling. The approach utilizes a wide range of chemical, physical, and genetic methods that exploit the resources available in different laboratories at two distinct institutions. The results of these studies will place us in the position to determine how signal input is recognized, integrated, and transduced in two-component response regulators. In addition, this specific signaling system regulates the only known natural vector for inter-Kingdom gene transfer and understanding this mechanism has already and will continue to widen the range of its use in biotechnology.

Grant: 2R01GM047446-09A1
Program Director: TOMPKINS, LAURIE
Principal Investigator: HELMANN, JOHN D
Title: B SUBTILIS ECF SIGMA FACTORS: ROLES AND REGULATION
Institution: CORNELL UNIVERSITY ITHACA ITHACA, NY
Project Period: 1992/05/01-2004/12/31

DESCRIPTION (adapted from the investigator's summary): The widespread emergence of antibiotic resistant bacteria poses a grave threat to our ability to manage and control infectious disease. While tremendous progress has been made in understanding the role of transmissible plasmids and high-level resistance genes in antibiotic resistance, the role and regulation of chromosomally-encoded determinants is less well understood. This project focuses on the genetically well characterized model organism *Bacillus subtilis*, to investigate the functional genomics of antibiotic resistance and responses. The close evolutionary relationship between *B. subtilis* and important human pathogens (especially *Staphylococcus aureus*, *Mycobacterium tuberculosis*, *Enterococcus*, and *Streptococcus*), allows knowledge gained in our system to be directly used in understanding the other. The goal of this project is to understand the role of alternative sigma factors in coordinating the genetic responses triggered by exposure of *B. subtilis* to antibiotics that target the cell envelope. Recently, the SigX and SigW regulators have been found to activate transcription of a large number of genes affecting the structure and function of cell surface polymers, antibiotic resistance mechanisms, and the production of antimicrobial peptides. Expression of these sigma factors is strongly induced by several clinically important antibiotics, including vancomycin and cephalosporins. To better define these genetic responses, and their roles in protecting the cell against antibiotics, two aims will be pursued. First, promoters controlled by each sigma factor will be identified and the rules that govern promoter selectivity will be explored. The identification of target promoters will reveal the complete set of genes (the regulon) activated by each sigma. The PI will define the overlap between the various regulons controlled by SigX, SigW and other sigma factors. This aim will include both proteomics and genomics based approaches. Second, the physiological roles of selected target genes will also be investigated. For this aim the PI will focus on those operons implicated in defense against antibiotics, modification of the cell envelope, or the production of antimicrobial compounds. In addition, the signaling pathways that control the expression of these regulons will be investigated. Although many different antibiotics can induce each regulon, it is likely that these antibiotics lead to the accumulation of common signaling molecules that are perceived by the anti-sigma factor which then releases the sigma factor. Genetic approaches have been devised to identify components of these signaling pathways. Together, these two aims will provide a unified picture of these two large regulons and their roles in *B. subtilis* physiology.

Grant: 2R01GM048707-09
Program Director: ANDERSON, JAMES J.
Principal Investigator: AUSUBEL, FREDERICK M PHD
CHEMISTRY:CHEMISTRY-
UNSPEC
Title: GENETIC ANALYSIS OF THE PLANT DEFENSE RESPONSE
Institution: MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA
Project Period: 1993/01/01-2004/12/31

Recent studies have shown that there are many common features of the plant and animal defense responses to pathogen attack, suggesting ancient origins. We have been using a genetic approach to study host defense responses in the model genetic organisms *Arabidopsis thaliana* and *Caenorhabditis elegans*. A set of *Arabidopsis* enhanced disease susceptibility (eds) mutants has been used to define a variety of defense response pathways that involve the low molecular mass signaling molecules salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). eds16, which accumulates much lower levels of SA in response to pathogen attack than wild-type plants, has been the most informative mutant in our current studies. The cloning of EDS16 has shown that it encodes isochorismate synthase (ICS), an enzyme involved in salicylic acid biosynthesis in bacteria, but not previously known to be involved in SA biosynthesis in plants. Our work with *C. elegans* primarily involves the broad host-range bacterial pathogen *Pseudomonas aeruginosa*, which is infectious in plants, insects, and mice and kills *C. elegans*. We have recently shown that it is possible to readily isolate *C. elegans* mutants that exhibit enhanced susceptibility to pathogen (esp) mutants, analogous to the *Arabidopsis* eds mutants isolated previously. We believe that direct experimental comparison of innate immunity in *Arabidopsis* and *C. elegans* will be highly synergistic. There are four specific aims. First, we propose to verify that EDS16 encodes an enzyme with ICS activity and to test the hypothesis that *Arabidopsis* has at least two SA biosynthetic pathways, one involving isochorismate and a second that involves phenylalanine as intermediates. Second we propose to clone and further characterize the *Arabidopsis* EDS5, EDR5, DDE1, EDS14, EDS15, and NPR2 defense-related genes and to determine the roles of these genes in defense-response signaling pathways. Third, we propose to clone and characterize the *C. elegans* esp-2 and esp-8 genes, which we have tentatively identified as defense-related genes, and to isolate additional *C. elegans* esp mutants. Finally, we propose to use genomic and reverse genetic approaches, including DNA microarray analysis, to identify *C. elegans* and *Arabidopsis* defense-related genes.

Grant: 2R01GM049338-09
Program Director: IKEDA, RICHARD A.
Principal Investigator: WALSH, CHRISTOPHER T PHD
Title: Enzymatic Assembly of Vancomycin Group Antibiotics
Institution: HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA
Project Period: 1993/04/01-2005/03/30

DESCRIPTION (Applicant's abstract): The glycopeptide antibiotics of the vancomycin family are potent antibacterial agents that can cure life-threatening bacterial infections by complexation of the D-Ala-D-Ala termini of peptidoglycan intermediates. The dramatic rise in resistance to vancomycin by gram positive pathogens (e.g., Vancomycin Resistant Enterococci) impels investigation into routes to more effective vancomycin analogs. Given the low likelihood of practical total syntheses, understanding of the enzymatic assembly of these nonribosomal peptide antibiotics offers prospect for subsequent reprogramming for combinatorial biosynthesis. This proposal deals with three phases of the enzymatic biosynthesis of vancomycin: (1) the beginning stages of the Nonribosomal Peptide Synthetase (NRPS) assembly line that makes the initial acyclic heptapeptide aglycone of vancomycin family members; (2) the post NRPS enzymatic tailoring of the heptapeptide, including oxidative crosslinking at the aryl side chains of residues 2,4,6,5 and 7, chlorination at 2 and 6, and glycosylation at 4 and 6; (3) the biogenesis of the 4-OH-phenylglycine incorporated at residues 4 and 5 of the core and 3,5-dihydroxyphenylglycine incorporated at residue 7. These aromatic amino acids are key sites for the crosslinking that produces the rigid cup-shaped architecture of the crosslinked core that enables recognition of the peptidoglycan termini and antibiotic action.

Grant: 2R01GM049530-18
Program Director: RHOADES, MARCUS M.
Principal Investigator: DUNNY, GARY M PHD MICROBIOLOGY, OTS
Title: Genetic Functions of an Enterococcal R Factor
Institution: UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN
Project Period: 1992/09/01-2005/03/31

DESCRIPTION (Verbatim from Applicant's Abstract): The enterococci have become prevalent as causes of nosocomial infections. The high incidence of resistance of these organisms to the most efficacious antibiotics (e.g., vancomycin) causes major problems in treating enterococcal infections, and the tremendous reservoir of enterococcal resistance determinants serves as a vector for the spread of these genes to other, more pathogenic bacterial genera. This research is focused on dissection of a mechanism of horizontal genetic transfer of the antibiotic resistance plasmid pCF10 in *Enterococcus faecalis*. The most novel feature of this transfer system is that the pCF10-containing donor cell perceives the presence of potential recipients in its vicinity by sensing a small peptide signal (a sex pheromone called cCF10) excreted by the recipients. The donor cell only expresses genes required for plasmid transfer when exogenous cCF10 is detected in the growth medium. Both the donor cells response to exogenous pheromone, and the negative control system that prevents expression of transfer functions in the absence of exogenous pheromone, are complex processes that have been studied in detail. Enterococci produce a variety of peptide pheromones, consisting of hydrophobic peptides 7-8 amino acids in length. Different families of plasmids each encode a highly specific response to a single cognate pheromone. When a single cell carrying multiple pheromone plasmids is exposed to one pheromone, only the corresponding plasmid is transferred, even though the sensing systems are quite similar for all the plasmids examined to date. In the next funding period, the focus of the experiments will be on the molecular and genetic basis for the specificity of the pheromone response. The specific aims are: 1) Determine the molecular basis for specificity of pheromone cCF10 interactions with PrgZ (the extracellular pCF10-encoded pheromone binding protein), and with PrgX (the putative intracellular receptor for cCF10 believed to comprise the molecular switch involved in the intracellular phase of pheromone induction. 2) Determine the molecular basis for specific abolition of endogenous cCF10 activity in pCF10-containing donor cells by PrgY (a pCF10-encoded membrane protein), and by iCF10 (a plasmid-encoded peptide inhibitor of cCF10). 3) Determine the molecular basis for the activity and specificity of a novel regulatory RNA, Qa in blocking expression of conjugation in uninduced cells via its interaction with PrgX and with the Qs RNA encoded in the positive control region of pCF10. 4) Begin an experimental analysis of the genetic and molecular basis for specificity of the downstream steps in pheromone induction. These steps include post-transcriptional activation of transfer gene expression, and conjugative DNA processing.

Grant: 2R01GM049700-07A1
Program Director: WOLFE, PAUL B.
Principal Investigator: CROOKE, ELLIOTT PHD
Title: Membranes in the Initiation of Chromosomal Replication
Institution: GEORGETOWN UNIVERSITY WASHINGTON, DC
Project Period: 1994/07/01-2005/03/31

DESCRIPTION: (Provided by applicant): Bacterial DNA replication is carefully controlled at the initiation stage, possibly by regulation of the essential activity of DnaA protein. The cellular membrane has long been hypothesized to be involved in chromosomal replication, with accumulating evidence that indicates membranes have a profound influence on DnaA protein. *E. coli* membrane liposomes can convert an inert form of purified DnaA protein into a replicatively active form. Characterization of the membranes has identified fluidity and acidic phospholipids as essential features. The importance of membrane fluidity and acidic phospholipids for this activation has been seen in vitro, and for DNA replication, in vivo. The long-term goal of this research is to elucidate the physiological significance of the influence of membranes on chromosomal replication. The research outlined here uses biochemical approaches with defined components, genetic and physiological studies, and cytolocalization techniques to directly test the hypothesis that the cellular membrane participates in the regulation of DnaA protein activity. The Specific Aims are to: Determine if the localizations of DnaA and the chromosomal origin to the site of initiation occur dependently or independently of each other, and if their proper localizations require acidic phospholipids. Using fluorescence microscopy, examine the importance of DnaA and acidic phospholipids in the proper localization of the chromosomal origin, and the importance of an intact origin and acidic phospholipids in the proper localization of DnaA protein. Generate strains in which the sole allele of *dnaA* is located on the chromosome and encodes for DnaA proteins with altered membrane-associating properties. Analyze how the mutant DnaA proteins affect cell cycle controlled chromosomal replication in vivo. Map the mutations in *dnaA* and examine the replication activities of the mutant proteins in vitro. Determine how the cellular locations of the mutant DnaA proteins may differ from that of wild-type DnaA protein. Isolate and identify the membrane component that inhibits acidic phospholipid reactivation of inert ADP-DnaA to active ATP-DnaA protein. Characterize the effect that deletion and overexpression of the gene(s) that encode the nucleotide release inhibitor have on the cell cycle controlled initiation of replication in vivo. This work should provide insight into the regulation of the initiation of DNA replication, which is a key control point in the prokaryotic cell cycle and in the determination of eukaryotic cellular quiescence or proliferation. Furthermore, knowledge gained from the proposed studies of DnaA protein may guide future investigations of how phospholipids act as regulators of enzymatic activities.

Grant: 2R01GM051126-06A1
Program Director: RHOADES, MARCUS M.
Principal Investigator: DYBVIG, KEVIN F PHD
Title: GENETIC RECOMBINATION IN MYCOPLASMAS
Institution: UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL
BIRMINGHAM
Project Period: 1995/01/01-2004/12/31

DESCRIPTION (ADPATED FROM APPLICANT'S ABSTRACT): Mycoplasmas are, widely distributed in nature and commonly produce disease in plants, insects, and animals, including man. Although the economic impact of these diseases is great, little information is available concerning mechanisms of pathogenesis and effective methods of control are unavailable. The surface properties of numerous mycoplasma species vary at high rate, about 10-3 per cell per generation. In the murine pathogen *Mycoplasma pulmonis*, high-frequency phenotypic variations involving changes in variable surface antigens (VI antigens) affect colony morphology, the susceptibility of the organism to mycoplasma viruses, and the adsorption of mycoplasmas to red blood cells. Most of the high-frequency phenotypic variations in *M. pulmonis* result from site-specific DNA inversions occurring in the *hsd* loci (encoding complex, phase-variable restriction and modification systems) and the *vsa* locus (encoding the phase-variable V-1 surface proteins). The finding of phase-variable restriction and modification systems is novel and challenges the notion that the function of restriction systems in bacteria is limited to the protection of cells from invasion by foreign DNA (phage infection). This challenge takes on added dimension by the observation that DNA inversions within *vsa* and within *hsd* apparently occur in concert. The long-range goals are to understand the molecular basis of mycoplasmal chromosomal and phenotypic variation and the role these variations have in disease pathogenesis. Completion of these goals is essential for the development of control measures for mycoplasmal diseases and into pathogenic mechanisms of bacterial diseases. The genome of *M. pulmonis* will have been fully sequenced by the end of 1999. This application proposes to capitalize on the forthcoming sequence information through the construction of a transposon library of *M. pulmonis* mutants. In aim 1, the chromosomal site of transposon insertion will be determined for each mutant to precisely identify the mutated gene. In aim 2, mutants with defects in putative DNA recombinases and V-I protein production will be examined to study the mechanisms of gene rearrangements and phenotypic switching.

Grant: 2R01GM051329-05
Program Director: CHIN, JEAN
Principal Investigator: TAMM, LUKAS K
Title: STRUCTURE AND FOLDING OF INTEGRAL MEMBRANE PROTEINS
Institution: UNIVERSITY OF VIRGINIA CHARLOTTESVILLE, VA
CHARLOTTESVILLE
Project Period: 1997/03/01-2005/02/28

Description: (From the applicant's abstract) About 30 to 40 percent of all human proteins are expected to be integral membrane proteins. Although the sequences of all genes coding for these proteins will be known in the very near future, the three-dimensional structures of only a few of them have been solved. Structure prediction from sequences may be simpler for membrane proteins than for soluble proteins because the ordered fluid structure of the lipid bilayer constrains the folding of membrane proteins. The major limitation of this approach is that many of the relevant fundamental forces that govern membrane protein folding are not yet known. The goal of this project is to study the structure and folding of membrane proteins by using the outer membrane protein A (OmpA) of E. coli as a model. Specifically, the structure of the transmembrane domain of this channel protein will be solved by NMR spectroscopy. Elementary lipid-protein and protein-protein interactions will be determined that direct the folding of this and, by extension, other membrane proteins. How different lipids modulate folding and to what extent the C-terminal domain contributes to the folding of OmpA will be investigated. Finally, the role of a candidate periplasmic chaperone protein will be examined. Since folding of membrane proteins is not an established field, an important component of the proposed research is to continue to develop new methods that are suitable for the characterization of intermediates of membrane protein folding.

Grant: 2R01GM052837-05A1
Program Director: PREUSCH, PETER C.
Principal Investigator: KRULWICH, TERRY A PHD
MICROBIOLOGY:BACTERIOLOGY
Title: Tet Efflux Proteins: Implications of Multiple Functions
Institution: MOUNT SINAI SCHOOL OF MEDICINE OF NEW YORK, NY
NYU
Project Period: 1996/08/01-2005/06/30

DESCRIPTION (provided by applicant): The Tet(L) and Tet(K) proteins are important Gram-positive antibiotic (tetracycline, Tc)-resistance proteins that possess three catalytic modes. They catalyze efflux of: (i) a tetracycline-divalent metal complex in exchange for a greater number of protons [Tc-Me²⁺/H⁺ antiport]; (ii) Na⁺ or K⁺ in exchange for a greater number of H⁺ [Na⁺(K⁺)/H⁺ antiport]; and (iii) Tc-Me²⁺, Na⁺ or K⁺ for a greater number of external K⁺ [Tc-Me²⁺ (Na⁺(K⁺)K⁺ antiport, i.e. net K⁺ uptake]. A comprehensive study of structure-function, physiological impact, and regulation pertaining to the multiple functions of these Tet proteins will contribute fundamental mechanistic insights into this class of transporters. In addition, these studies will enhance approaches to rational design of inhibitors and to assessments of selective pressures, other than Tc, that enhance retention of the tet genes. Specific Aim #1 is to develop structure-function information about Tet(L) and Tet(K) through integrated use of biochemical and site-directed mutagenesis approaches. Deployment of a comprehensive array of vesicle and proteoliposome assays of the three catalytic activities will: probe a ping-pong model and hypothesized involvement of occluded transition states in the catalytic cycles; clarify the coupling stoichiometry, and whether K⁺ ions can completely substitute for H⁺; extend the analysis of the critical functions of the Motif C region of the transporter in ion-coupling and substrate preference. Selected mutant Tet proteins will also be examined for their effects on competitive fitness of the natural host relative to wild type Tet(L) or Tet(K). Using a combination of molecular, genetic and biochemical approaches, the oligomeric structure of Tet(L) will be examined. Specific Aim #2 is the continuation of the structural biology initiative which is currently focused primarily on further improvement of the quality and diffraction properties of 3D crystals of Tet(L). Crystals of sufficient quality, perhaps stabilized in a single conformation by inhibitors, substrates or conformationally sensitive monoclonal antibodies, will be analyzed by x-ray diffraction. A parallel track is also to be pursued on 2D crystals followed by cryoelectron microscopy. These studies, conducted while the longer-term 3D effort continues, is expected to provide lower resolution structural information of interest. Specific Aim 3 will: clarify the basis for different phenotypes of tet(L) mutants; will further test the novel translational reinitiation model for tet(L) regulation; and will probe additional, Na⁺- and pH related regulation.

Grant: 2R01GM052882-15A1
Program Director: MARINO, PAMELA
Principal Investigator: RICK, PAUL D PHD
Title: Biosynthesis of Enterobacterial Common Antigen
Institution: HENRY M. JACKSON FDN FOR THE ADV ROCKVILLE, MD
MIL/MED
Project Period: 1984/07/01-2005/03/31

DESCRIPTION: (Adapted from the Investigator's abstract): The long-term goals of this research are to gain a more complete understanding of the function of the outer membrane (OM) of gram-negative bacteria and the mechanisms involved in its biogenesis. As an approach to these goals, we have investigated the biochemistry and genetics of enterobacterial common antigen (ECA) synthesis and assembly in *Escherichia coli*. In addition, we have investigated the function of ECA in *E. coli*, *Shigella flexnerii*, and *Salmonella enterica* serovar typhimurium. ECA is an OM glycolipid that is unique to the Enterobacteriaceae, and it is present in all members of this family. In spite of the ubiquitous occurrence of ECA in gram-negative enteric bacteria, the function of ECA has remained unknown. Our previous endeavors to study ECA synthesis and assembly have been facilitated by the isolation and characterization of mutants defective in these processes. The characterization of these mutants has resulted in the identification of biosynthetic intermediates involved in ECA synthesis that have, in turn, led to the development of in vitro and in vivo experimental systems to demonstrate specific enzymatic steps in ECA assembly. Nevertheless, several important steps in the assembly of ECA remain to be established. We propose to continue this combined biochemical and genetic approach to complete the characterization of the assembly process. In addition, we have obtained exciting new data that strongly supports the conclusion that ECA plays an important role in the mechanism responsible for the resistance of gram-negative enteric bacteria to bile salts. Thus, the specific aims for the requested period of support are: (i) identification of the genetic determinant of ECA polysaccharide chain elongation in *E. coli* K-12, and biochemical characterization of the polymerization mechanism, (ii) determination of the role of *o416* of the *E. coli* *wec* gene cluster in ECA assembly, (iii) isolation of *E. coli* mutants defective in the synthesis of the ECApG polysaccharide-aglycone linkage and biochemical characterization of the mechanism involved in linkage formation, and (iv) determination of the role of ECA in the resistance of gram-negative enteric bacteria to bile salts. It is anticipated that these studies will provide valuable insights into the assembly of other important membrane glycolipids and polysaccharides. Such information will also provide a rationale for the development of new antimicrobial agents.

Grant: 2R01GM052964-06
Program Director: SCHWAB, JOHN M.
Principal Investigator: ROMO, DANIEL PHD
Title: SYNTHETIC/MECHANISTIC STUDIES OF BIOACTIVE MARINE AGENTS
Institution: TEXAS A&M UNIVERSITY SYSTEM COLLEGE STATION, TX
Project Period: 1995/08/01-2004/07/31

DESCRIPTION: (Principal Investigator's Abstract) The objectives of the proposal include total synthesis and biomechanistic studies of the marine natural products pateamine A, palau'amine, gymnodimine, and phakellin which possess novel structures and exhibit potent physiological effects. Natural products that have strong and specific cellular effects have proven to be powerful biochemical probes for dissecting molecular mechanisms of signal transduction pathways involved in various cellular functions. Pateamine A and palau'amine are potent immunosuppressive agents that promise to be useful biochemical probes for elucidating cellular events involved in the immune response. Thus, these natural products may potentially lead to new therapeutic targets for not only organ transplantation therapy, but also diabetes, multiple sclerosis, and rheumatoid arthritis. Gymnodimine is a potent marine toxin that possesses an unusual spirocyclic imine moiety. Its precise molecular mechanism of toxicity has not been elucidated although it appears to be unique. Marine toxins have proven useful for the study of ion channels, protein phosphatases, and neurotransmitter receptors. Thus, gymnodimine promises to be a useful biochemical probe for studies of neuronal function. Phakellin has been proposed to be responsible for the powerful antibiotic effects observed in extracts from the marine sponge *Phakellia flabellata*. Considering the rise in antibiotic resistance in recent years, the search for novel antibiotics has intensified. The utility of the tetracyclic guanidine structure in phakellin and congeners as new antibiotics will be assayed. A naturally conjoined objective in our total synthesis efforts is the development of new synthetic methods and strategies for the concise synthesis of these targets. In this regard, several new methods and strategies including formylation of vinyl halides, Hantzsch thiazole synthesis with unactivated bromoketones, Diel-Alder reactions of alpha-exomethylene lactams and vinyl imidazolidinones, a single pot lactam to cyclic imine synthesis, an intramolecular chlorination/1,2 shift sequence, and the use of latent pyrroles will be studied. The synthetic products resulting from this research will enable us and our collaborators Prof. Jun Liu (MIT) and Dr. Chris Miles (AgResearch) to address questions of biological and thus health significance.

Grant: 2R01GM053008-08A1
Program Director: SOMERS, SCOTT D.
Principal Investigator: WANG, PING MD MEDICINE
Title: MODULATION OF HEPATOCELLULAR FUNCTION IN EARLY SEPSIS
Institution: UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL
BIRMINGHAM
Project Period: 1995/08/01-2002/08/31

Our recent studies have indicated that depression in hepatocellular function occurs very early after the onset of polymicrobial sepsis (as induced by cecal ligation and puncture). This depression is associated with increased norepinephrine (NE) production and Kupffer cell (KC)-derived TNF-alpha release. The gut appears to be the major source of NE release during early sepsis, as evidenced by the increased intestinal tyrosine hydroxylase and dopamine beta-hydroxylase (enzymes responsible for NE biosynthesis) and significantly higher levels of NE in portal than in systemic blood. Administration of NE via the portal vein or in the isolated perfused liver preparation increases TNF-alpha release and depressed hepatocellular function. In contrast, alpha2-adrenergic antagonists rauwolscine or yohimbine attenuate TNF-alpha production and prevent hepatocellular depression in early sepsis as well as after NE infusion. Enterectomy prior to the onset of sepsis also reduces circulating levels of NE and TNF-alpha and prevents hepatocellular depression. In addition, alpha2-adrenergic agonists NE or clonidine increases TNF-alpha release in vivo as well as in isolated KC. Moreover, the number and affinity of alpha2-adrenoceptors in KC appear to be increased in early sepsis. We therefore hypothesize that the increased gut-derived NE during early sepsis upregulates TNF-alpha production in KC through an alpha2-adrenoceptor pathway and is responsible for producing hepatocellular depression. Since administration of glycines at the early state of sepsis decreases TNF-alpha release and protects hepatocellular function, we further hypothesize that modulation of KC responsiveness to NE by reducing intracellular calcium in KC and/or reducing gut-derived NE release are the mechanisms responsible for the beneficial effect of this amino acid on hepatocellular function during sepsis. Studies are proposed to determine the mechanisms: 1) responsible for the increased NE biosynthesis in the gut during the early stage of sepsis; 2) by which gut-derived NE upregulates TNF-alpha production in KC and depresses hepatocellular function in sepsis; 3) responsible for the beneficial effect of glycines on hepatocellular function under such conditions. The proposed studies should provide useful information not only for better understanding the precise mechanisms responsible for hepatocellular depression during the early stage of polymicrobial sepsis, but also for intercepting such depression and preventing subsequent multiple organ dysfunction and mortality.

Grant: 2R01GM053069-05A1
Program Director: JONES, WARREN
Principal Investigator: WOODARD, RONALD W
Title: Mechanism of KDO 8-P and DAH 7-P Synthase
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 1996/07/01-2005/03/31

DESCRIPTION: (Provided by Applicant) Health care providers are challenged daily by an increasing resistance of pathogenic bacteria to their antibacterial arsenal. To overcome this problem, it is necessary to design new and innovative antibiotics with totally different modes of action so that, no cross-resistance with present agents should occur. Most antimicrobial drugs act by inhibiting key enzymes in the biosynthesis of macromolecular molecules necessary for viability of the microorganism. Success in this type of approach necessitates a thorough understanding of the enzyme(s) at the molecular level. The goal of this work is to collect mechanistic information on the enzymes 3-deoxy-D-mannoo-octulosonate 8-phosphate and 3-deoxy-D-arabino-heptulosonate 7-phosphate synthase. The information will prove useful in the design of selective inhibitors of these unique enzymes, namely a new generation of mechanistically diverse antibiotics. The goals of this project are to establish 1. The mechanism for the formation of 3-deoxy-D-manno-octulosonic 8-phosphate (KDO 8-P) from arabinose 5-phosphate (A 5-P) and phosphoenolpyruvate (PEP) catalyzed by the enzyme KDO 8-P synthase (EC 4.1.2. 16), an enzyme involved in the biosynthesis of the lipid A portion of the lipopolysaccharide region of the cell envelope of gram-negative bacteria, 2. The mechanism for the formation 3-deoxy-D-arabino-heptulosonate 7-phosphate (DAH 7-P) from erythrose 4-phosphate (E 4-P) and PEP catalyzed by the enzyme DAH 7-P synthase [EC 4.1.2.15], the enzyme that catalyzes the first committed step in the biosynthesis the aromatic amino acids and various aromatic secondary metabolites. The specific aims focus on the use of diverse techniques to "visualize" the potential tetrahedral intermediate. These methods include a rapid mixing, pulsed-flow ESIMS technique to confirm the formation of a reaction intermediate(s) and rotational-echo double-resonance NMR experiments of sub-zero substrate entrapped in enzyme to observe the intermediate. A rapid temperature quench methodology will be developed to isolate the potential intermediate(s) for NMR structural studies. Multinuclear NMR analysis of the interaction of the synthases with various labeled substrate analogues will be utilized to observe abortive intermediates and substrate analogs designed to "stabilize" this potential abortive intermediate(s) will be used to further understand the mechanisms of these reactions. The role of the metal ion will also be investigated. Site-directed mutagenesis studies, based on x-ray crystallographic data, will be exploited to gain further insight into the contribution of enzyme functionalities to substrate binding, monomer interface interactions and to the mechanism of the enzyme.

Grant: 2R01GM053158-07
Program Director: WOLFE, PAUL B.
Principal Investigator: ROSENBERG, SUSAN M
Title: MOLECULAR MECHANISM OF ADAPTIVE MUTATION IN E.COLI
Institution: BAYLOR COLLEGE OF MEDICINE HOUSTON, TX
Project Period: 1995/09/30-2005/07/31

DESCRIPTION (provided by the applicant): Spontaneous mutations drive evolution, and are also important in many medical contexts including the origin and progression of some cancers, development of resistance to chemotherapeutic and antibiotic drugs, and evasion of the immune response by pathogens. In the past decade, understanding of spontaneous mutation has expanded with the discovery that some mutational processes are not purely random, but can be induced by environmental conditions favoring genetic change. These "adaptive" (or stationary-phase) mutations occurring in slowly-growing or non-growing cells, only after the cells are exposed to a growth-limiting environment, and can confer a growth advantage in that environment. Little is known about the mechanisms of stationary-phase mutation in most of the assays in which they have been reported in bacteria and yeast. However, in our system, reversion of a lac frame-shift mutation in *Escherichia coli*, aspects of the molecular mechanism is becoming apparent, and indicate a novel mutagenic route. The mechanism involves DNA double-strand break homologous recombination, DNA synthesis and polymerase errors, a transient depression of post-synthesis mismatch repair capacity, and the SOS DNA damage response. The mutations are not directed in a Lamarckian manner to lac gene, and are not specific to the episomal replicon that carries lac. Some, or all of the cells that acquire a Lac⁺ adaptive mutation have experienced transient hyper-mutability, affecting genes throughout their genomes. This project is aimed at providing a complete description of the molecular mechanism of stationary phase mutation in the lac system. Understanding the molecular mechanism will provide a valuable new model for mutation in non-dividing and slowly growing cells. This model may apply to many systems previously assumed to follow the rules of Luria/Delbruck growth-dependent mutation, occurring randomly before selective environments are encountered.

Grant: 2R01GM053210-04
Program Director: SHAPIRO, BERT I.
Principal Investigator: WEIS, ROBERT M PHD
Title: LIGAND & SUBUNIT INTERACTIONS OF TRANSMEMBRANE RECEPTORS
Institution: UNIVERSITY OF MASSACHUSETTS AMHERST, MA
AMHERST
Project Period: 2001/03/01-2002/02/28

DESCRIPTION: (adapted from applicant's abstract) Transmembrane signaling is a process common to all living cells. The program of work described this application is designed to discover how extended interactions between receptor subunits are important for transmembrane signaling in the chemosensory pathway of *Escherichia coli*. Since these interactions appear to be an essential part of the signaling process, a deeper understanding of them may lead to insights of fundamental importance to the mechanism of trans-bilayer communication in all cells. The available evidence suggests that the trans-bilayer communication can, under some circumstances, mutually stabilize the ligand binding interaction on both sides of the membrane, and in other situations the communication is predicted to be mutually destabilizing. This concept will be put to the test with measurements of ligand binding and protein-protein interaction between the receptor, and its various ligands. The experiments are designed to test the influence of one ligand on the binding of others. In addition to an equilibrium investigation of the system, the dynamics of subunit assembly and disassembly will be more thoroughly studied, since recent results indicate that the assembly/disassembly process is a ligand-catalyzed process, and can occur on a time scale relevant for signaling. These data will be used to distinguish two models of transmembrane signaling, one in which the chemoreceptors and the associated signaling proteins are in a stably-assembled complex, and another in which the receptor complexes dynamically associate and dissociate on the signaling time scale.

Grant: 2R01GM054233-06
Program Director: WEHRLE, JANNA P.
Principal Investigator: RALEIGH, DANIEL P PHD CHEMISTRY
Title: Folding And Stability Of Protein Domains and Sub-Domains
Institution: STATE UNIVERSITY NEW YORK STONY STONY BROOK, NY
BROOK
Project Period: 1996/05/01-2005/04/30

DESCRIPTION (provided by applicant): This proposal describes experimental studies of protein folding in vitro. Elucidating how the amino acid sequence determines structure; i.e., the protein folding problem, is a central issue in modern structural biology. Two recent developments have made this problem particularly timely. Massive sequencing efforts have led to an explosion in Dr. Raleigh's knowledge of the primary sequence of proteins. Unfortunately, it is still impossible to predict structure from sequence. In principle, a more thorough understanding of the folding process will aid efforts to decipher the code that links sequence and structure. A detailed understanding of the folding process will also aid efforts to rationally modify proteins to enhance desired properties. The growing realization that protein misfolding and aggregation play a role in a number of different diseases has also focussed attention on the folding problem. A lack of structural specificity or a breakdown in the cooperativity of folding can have severe physiological consequences, and partially folded states play an important role in pathophysiological protein aggregation. This proposal focuses on the experimental investigation of several key issues in protein folding: (1) the structural heterogeneity of partially folded states of proteins (2) the origins of cooperativity in folding (3) the role of subdomains in folding (4) the role of overall chain topology and specific interactions in rapid folding. The molten globule state is a key intermediate in folding. A protein dissection approach in combination with studies of mutants will be used to pinpoint key regions of the molten globule state of alpha-lactalbumin. It is now recognized that proteins can populate a wide range of different types of partially folded states. Comparative studies of different members of the alpha-lactalbumin/lysozyme family will provide information about the origins of the different types of partially folded states. Kinetic experiments will elucidate the steps involved in the cooperative formation of the native state from the molten globule. Kinetic experiments with three small, rapid folding, helical proteins will provide information about the fundamental steps in rapid folding.

Grant: 2R01GM054246-05A1
Program Director: LEWIS, CATHERINE D.
Principal Investigator: VARUGHESE, KOTTAYIL I PHD
Title: Structure of Sporulation Proteins in *Bacillus subtilis*
Institution: SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA
Project Period: 1997/01/01-2005/03/31

Bacteria, fungi and plants recognize and respond to a multitude of environmental, metabolic and cell cycle signals through two-component signal transduction system and phosphorelays. These systems regulate a variety of genes and operons ranging from essential functions and virulence determinants to development and the ripening of fruit. Signal input activates an ATP-dependent autophosphorylation of a histidine residue of a kinase and signal propagation in these systems involves His-Asp phosphotransfer discovered, that of sporulation in bacteria, has been the subject of extensive genetic and biochemical studies. Structural analysis of the components of the pathway have shown how these signaling proteins interact and have given a first view of what residues determine specificity of molecular recognition. The objectives of the present proposal are to elucidate in detail the nature of molecular recognition, molecular specificity, and the mechanism of phosphotransfer in the phosphorelay using crystallographic techniques in conjunction with biochemical and genetic analyses. Two areas of focus will be the structure of the ultimate transcription factor, Spo0A, and its interaction with DNA and the structure of the signal-sensing histidine kinase that regulates phosphoryl input in the phosphorelay. The objective of this latter aim is to understand the mechanism by which signal ligand interaction activates kinase activity. Through these studies the complete mechanism of the pathway of signal transduction from signal ligand binding to gene activation will be understood.

Grant: 2R01GM054666-06
Program Director: DEATHERAGE, JAMES F.
Principal Investigator: SHI, WENYUAN
Title: Intercellular signaling in *Myxococcus xanthus*
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 1996/08/01-2005/07/31

Myxococcus xanthus cells exhibit coordinated cell movements on a solid surface during vegetative growth and multicellular fruiting body formation. These complex social behaviors make this bacterium an excellent model system for studying intercellular signaling and microbial development. The focus of our research is to understand the mechanical and physiological basis of social gliding motility (S-motility) and the chemotactic control of motility during fruiting body formation. During the last grant period, we discovered some important molecular functions of two cell surface appendages that are required for social motility: the type IV pili and fibrils. Based on these findings, we hypothesize that directed motility in *M.xanthus* involves the control of pilus switching frequency by the *frz* chemosensory system. We also hypothesize that fibril, a self-generated extracellular matrix material, is a major chemoattractant for *M. xanthus* and provides an important signal for fruiting body formation in *M. xanthus*. We propose the following specific aims to test these hypotheses: 1. to obtain direct physical evidence of pilus dependent motility and study its interaction with the *frz* chemosensory system; 2. to obtain direct visual evidence of fibril-guided chemotactic movement during fruiting body formation; 3. to identify molecular components involved in self-generation of fibril gradient. The studies will help us to understand the molecular mechanisms of social swarming, social hunting, intercellular signaling, and fruiting body formation. It will also provide a molecular understanding of gliding motility and the evolution of bacterial motility/chemotaxis systems. Since the S-motility and aggregation of *M. xanthus* are very similar to twitching motility and biofilm formation in pathogenic bacteria like *Pseudomonas* and *Neisseria*, the studies may also provide clues for further molecular characterization of these events, leading to new treatments against these pathogenic bacteria.

Grant: 2R01GM054775-04A1
Program Director: SOMERS, SCOTT D.
Principal Investigator: JAMES, J H PHD
ANATOMY/PHYSIOLOGY
Title: MUSCLE Lactate Production in Sepsis
Institution: UNIVERSITY OF CINCINNATI CINCINNATI, OH
Project Period: 1997/05/01-2005/03/31

The long-term objective of this work is to understand the changes in metabolism in sepsis in order to improve the therapy of septic patients. Sepsis results in a hypermetabolic state in which many aspects of carbohydrate metabolism are abnormal: enhanced peripheral glucose uptake and utilization, hyperlactacidemia, increased gluconeogenesis, depressed glycogen synthesis, glucose intolerance and insulin resistance. Traditionally, high circulating lactate concentration has been interpreted as tissue hypoxia or mitochondrial dysfunction. However, therapy to improve tissue perfusion does not always prevent lactate accumulation. Current understanding of energy metabolism cannot explain persistent glycolysis by well-oxygenated tissues. High epinephrine levels in sepsis may cause the characteristic changes in carbohydrate metabolism through stimulation of the Na⁺-K⁺ pump in skeletal muscle. The stimulation of glycogen breakdown and lactate production in muscle by epinephrine may be closely tied to stimulation of the Na⁺, K⁺-ATPase, implying that muscle energy metabolism takes place in separate glycolytic and oxidative compartments. ATP consumption by the Na⁺, K⁺-ATPase appears to be the primary influence on ATP production in the glycolytic compartment. This proposal aims to explore these relationships in greater detail, both in septic and nonseptic rats. Studies will combine in vivo and in vitro assessments of Na⁺-K⁺ pump activity, membrane recruitment, glycolysis, glycogenolysis, ATP content and membrane permeability to Na⁺ and K⁺. The central role of epinephrine in sepsis-induced metabolic derangements will be examined in two ways (i) chronic infusion of epinephrine using implantable minipumps and (ii) chronic infusion of the beta-adrenergic blockers in sepsis. Studies in vitro will examine the persistence of epinephrine's effects on glycolysis and Na⁺, K⁺-ATPase activity after beta-blockade has occurred. Results of these studies will clarify metabolic relationships that are important both in health and disease.

Grant: 2R01GM054803-05
Program Director: PREUSCH, PETER C.
Principal Investigator: BLACKBURN, NINIAN J
Title: X-ray Spectroscopic Studies on Heme-Copper Oxidases
Institution: OREGON GRADUATE INSTITUTE SCIENCE & BEAVERTON, OR
TECH
Project Period: 1997/05/01-2002/04/30

DESCRIPTION: (provided by applicant) The central role of heme-copper oxidases in biological energy production has made them the targets of intense study in many laboratories across the globe. Since the crystal structures have now defined the coordination of the redox centers and the identity of proton pumping channels, attention has focused on determining mechanistic details. Unraveling these mechanisms has required application of specialized spectroscopic and biophysical techniques. Our contribution has been the application of X-ray absorption spectroscopy (XAS) to uniquely define the coordination of the copper centers in a number of derivatives of different members of the oxidase family. The lack of optical or magnetic signals from CuB makes XAS particularly relevant to testing proposed structures for resting and intermediate states of the binuclear center. In this renewal application, we will extend our studies to include novel members of the oxidase family and catalytic intermediates. First, we will investigate resting states of the CuB centers of quinol oxidases from *Bacillus subtilis*, *Acidianus ambivalens*, *Thioalcalomicrobium aerophilum*, and a CuA-deleted mutant of *Thermus thermophilus*. The reduced forms will be examined carefully for evidence of functionally important histidine dissociation and chloride ligation as recently observed for the b(03) enzyme. We will next determine the structures of key intermediates in the reaction pathway, again searching for evidence of histidine dissociation from CuB proposed to be an important element of proton pumping. These data will be backed up by studies on inorganic and engineered protein models for cytochrome oxidase. Finally, we will begin to explore the mechanism of interaction of cytochrome oxidase with recently discovered metallochaperones, which are believed to be essential for proper maturation of the intact oxidase in the membrane.

Grant: 2R01GM055005-05
Program Director: SOMERS, SCOTT D.
Principal Investigator: SPOLARICS, ZOLTAN MD
Title: GLUCOSE-6-PHOSPHATE DEHYDROGENASE DEFICIENCY AND SEPSIS
Institution: UNIV OF MED/DENT NJ NEWARK NEWARK, NJ
Project Period: 1997/02/01-2005/01/31

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human genetic polymorphism. A clinically significant variant is the type A deficiency present in 10-12% of African Americans. G6PD is a major supporter of cellular redox status. Our clinical investigations on young African American trauma patients revealed that after severe trauma, the type A- G6PD deficiency predisposes to the development of bacteremic sepsis, an augmented inflammatory response and worsens anemia. The defect also alters the trauma-induced monocyte responses. We will investigate the impact of the defect on the sepsis-associated multiple organ dysfunction/failure and mortality after major trauma. We will test if a compromised RBC function and an altered activation status of the reticulo-endothelial/monocuclear phagocyte system contribute to the adverse clinical effects of the deficiency. The study use G6PD deficient and nondeficient human endothelial cells (HUVEC), monocytes and neutrophils and an animal model. Hypotheses: 1: After severe trauma, the sepsis-associated mortality is greater in G6PD deficient patients than non-deficient patients. The adverse clinical course of G6PD deficient patients is associated with a diminished production of anti- inflammatory cytokines by monocytes, increased hemolysis, and decreased RBC deformability. The prospective cohort study will compare the clinical parameters in G6PD deficient and nondeficient patients after major trauma (ISS>13) and the time dependent changes in cytokine patterns representative of the proinflammatory/anti-inflammatory balance in leukocytes. Alterations in RBC deformability, lipid peroxidation, nitrosylated protein, nitroso-thiol and glutathione content will also be followed. 2: After oxidative stress, G6PD deficient endothelial cells and monocytes display an augmented pro-inflammatory response compared to non-deficient cells. Activation of redox-dependent transcription factors (NFkB,AP1,SP1) and redox status (GSH/GSSG) and the accompanying changes in cytokine production will be determined after ischemia- reoxygenation or chemically-induced oxidative stress in deficient and non-deficient HUVEC and monocytes. 3. G6PD deficiency diminishes the antioxidant capacity of endothelial cells more severely than the antioxidant capacity of phagocytes which results in an enhanced phagocyte-mediated endothelial dysfunction. Phagocyte-mediated endothelial cell apoptosis/injury will be measured in co-cultures using deficient or non-deficient cells. Using specific inhibitors of G6PD in an animal model will test endothelial cell dysfunction. The studies will elucidate if the adverse clinical effects of G6PD deficiency are manifested in elevated mortality and worsening organ dysfunction after injuries. The study will reveal important and novel information on the potential mechanisms, including RBC dysfunction, monocyte activation, and the role of redox regulated gene expression that is responsible for the adverse clinical effects of G6PD deficiency in trauma patients.

Grant: 2R01GM055073-04
Program Director: LEWIS, CATHERINE D.
Principal Investigator: HATFIELD, G WESLEY PHD BIOLOGY NEC:BIOL
NEC-UNSPEC
Title: BRANCHED CHAIN AMINO ACID BIOSYNTHESIS IN E COLI
Institution: UNIVERSITY OF CALIFORNIA IRVINE IRVINE, CA
Project Period: 1998/05/01-2005/04/30

DESCRIPTION: (Adapted from the Investigator's abstract): Our current understanding of gene regulation in Escherichia coli suggests three hierarchical levels of control: 1) global control of basal level gene expression by chromosome structure; 2) global regulatory protein-mediated control of stimulons and regulons; and, 3) operon specific controls. The first level of control is exemplified by the DNA-supercoiling-dependent mechanisms described for the coordination of basal level expression of operons of the *ilv* regulon. It has been shown that these controls can be influenced by the topological structure of the chromosome, and that DNA architectural proteins such as IHF are able to modulate the formation and location of these structures. The experiments described in this proposal are designed to further characterize this first level of global gene regulation. It is proposed that regulation of gene expression by chromosome structure is influenced by the energy charge of the cell, which in turn is influenced by nutritional and environmental conditions that require transitions from one growth state to another. To test this idea, computational and genomic methods will be employed. DNA arrays will be used to determine differential gene expression profiles, and cellular energy charge and DNA supercoiling levels will be monitored during aerobic to anaerobic growth transitions in the presence and absence of IHF. Computational predictions of IHF binding sites and predictions of the topological state of the E. coli chromosome will be used to analyze these data. It is expected that these experiments will identify additional operons that respond to energy charge and DNA supercoiling-mediated signals for further characterization. These experiments will further provide a wealth of information for future studies concerning the operon specific and global regulation of carbon and energy metabolism genes during aerobic to anaerobic growth transitions.

Grant: 2R01GM055194-05
Program Director: SOMERS, SCOTT D.
Principal Investigator: HOTCHKISS, RICHARD S MD ANESTHESIOLOGY
Title: Cellular Mechanisms of Sepsis and Organ Failure
Institution: WASHINGTON UNIVERSITY ST LOUIS, MO
Project Period: 1997/09/30-2005/08/31

Sepsis is the leading cause of death in many intensive care units. It is the 12th leading cause of death in the United States and the incidence is increasing rapidly because of the growing number of immunosuppressed patients. Although the general consensus has been that sepsis represents a disorder due to uncontrolled inflammation, the failure of numerous anti-inflammatory strategies has led to a rethinking of this concept. Recent evidence in both animal models and in clinical studies of patients dying of sepsis and multiple organ failure indicates that sepsis causes extensive apoptotic cell death of lymphocytes throughout the body. This loss in lymphocytes may impair the ability of patients to eradicate infection and predispose to secondary infections. Interestingly, patients with inherited defects in lymphocytes have infections with many of the same organisms as patients in the intensive care unit. Furthermore, these patients also have difficulty eradicating infections. Recent studies in which lymphocyte apoptosis was prevented by two independent methods, i.e., overexpression of the anti-apoptotic protein Bcl-2 or administration of drugs that prevent cell death proteases (caspase inhibitors), have shown a marked improvement in sepsis survival. The focus of this investigation is to determine, using transgenic mice models and adoptive transfer of lymphocytes, which lymphocyte subsets, i.e., CD4 helper T cells, CD8 cytotoxic T cells, or B cells, are critical for sepsis survival. The changes in lymphocyte effector function that occur in survivors vs. non-survivors will also be determined. Two other potential mechanisms of immune suppression in sepsis will be examined including: 1) effects of apoptotic cells to induce anti-inflammatory responses and, 2) a shift in the pattern of cytokine expression from the pro-inflammatory Th1 to the anti-inflammatory Th2.

Grant: 2R01GM055425-18
Program Director: CHIN, JEAN
Principal Investigator: ROSEN, BARRY P
Title: Mechanism of an ATP-coupled arsenical pump
Institution: WAYNE STATE UNIVERSITY DETROIT, MI
Project Period: 1997/05/01-2005/08/31

Resistance to arsenic and antimony is widely spread in both gram-positive and gram-negative bacteria. The best-characterized system encoded by the clinically isolated resistance factor plasmid R773 in *E. coli* confers resistance against oxyanions of arsenic (arsenite and arsenate) and antimony (antimonite). The *ars* operon codes for two regulatory (*ArsR* and *ArsD*) and three structural (*ArsA*, *ArsB* and *ArsC*) proteins. Resistance correlates with active extrusion of arsenite from the cell by a primary pump. The *arsA* gene product is an ATPase that serves as the catalytic subunit of the pump. *ArsA* is a 63-kDa peripheral membrane protein that catalyzes As(III)/Sb(III)-stimulated ATP hydrolysis. *ArsA* has two homologous halves, A1 and A2, connected by a short linker. Each has a consensus nucleotide binding site (NBS), and both NBS are required for activity. *ArsA* binds to *ArsB*, which is the ion-conducting subunit of the pump. *ArsB* is a 45-kDa integral membrane protein that spans the inner membrane 12 times. *ArsB* has a novel dual mode of energy coupling depending on its association with *ArsA*. Arsenic efflux bacteria can be catalyzed by either *ArsB* alone functioning as a secondary transporter or by the *ArsAB* complex, functioning as a transport ATPase. Our overall goal is elucidation of the molecular mechanism of the *ArsAB* pump. Analysis includes the following specific aims: 1. Structure and function of the *ArsA* ATPase: The function and properties of the metal binding site, nucleotide binding sites and signal transduction sites of *ArsA* will be examined. The composition and function of residues that form the interface between A1 and A2 will be determined. 2. Catalytic mechanism of the *ArsA* ATPase will be examined using single tryptophan *ArsA* mutants as spectroscopic probes, by isotope trapping and by vanadate trapping and cleavage experiments. 3. Structure of the *ArsAB* pump: Residues that form the sites of interaction of the *ArsA* and *ArsB* subunits will be identified. The stoichiometry of the *ArsA* and *ArsB* subunits in the pump will be determined.

Grant: 2R01GM055430-10
Program Director: CHIN, JEAN
Principal Investigator: LEVY, STUART B MD
Title: EFFLUX-MEDIATED RESISTANCE TO TETRACYCLINES
Institution: TUFTS UNIVERSITY BOSTON BOSTON, MA
Project Period: 1996/07/01-2004/11/30

DESCRIPTION (Adapted from the Applicant's Abstract): Efflux has become increasingly recognized as a mechanism of resistance to antibiotics and other drugs since the investigators' discovery of the tetracycline efflux protein Tet some 20 years ago. Today, a number of Tet efflux proteins (related by primary sequence) have been described which have two halves, each six alpha-helical transmembrane domains, separated by a cytoplasmic loop and specify an exchange of a tetracycline/cation complex for a proton. Active efflux is one of two major mechanisms of tetracycline resistance which have curtailed the clinical effectiveness of this important family of antibiotics. Studies in the Principal Investigator's laboratory focus on the biochemical and molecular basis of efflux by the 43kD class B Tet protein specified by Tn10. The protein has been purified in detergent as a polyhistidine fusion. Recently, it has been successfully crystallized for 2D analysis. This proposal seeks to determine the tertiary structure of the TetA protein through crystallization methods, combining 2D and 3D efforts, by cysteine cross-linking of site-directed cysteine mutant residues, and by genetic analysis of suppressor mutations. In a second aim, they shall pursue findings that implicate the interdomain loop in the function of the Tet protein, using mutagenesis of the loop and cysteine cross-linking studies to examine how the loop interacts with other domains of the Tet protein. A third aim will continue their work on radiolabeled photoaffinity tetracycline analogs to identify the substrate-binding region in the Tet protein. This approach will be complemented by studies that use iron as the cation for tetracycline efflux and the Fenton reaction to produce peptide bond cleavage at substrate binding sites in the protein. These studies pursue further the molecular basis for antiport by the drug efflux protein Tet. Advances in understanding of the Tet protein will enhance our knowledge of other, similar proteins in the major facilitator family of transport proteins which should have broad impact in the area of drug treatment of infectious disease and cancer.

Grant: 2R01GM055434-17A1
Program Director: ANDERSON, JAMES J.
Principal Investigator: SAIER, MILTON H PHD
Title: ATP-Dependent Protein Kinases in Gram-Positive Bacteria
Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA
Project Period: 1984/12/01-2005/06/30

Low G+C Gram-positive bacteria possess the phosphoenolpyruvate:sugar phosphotransferase system (PTS) which transports and initiates the metabolism of its sugar substrates and influences the processes of catabolite repression (CR) and catabolite activation (CA). We and others have shown that a metabolite-activated, ATP-dependent protein kinase, PtsK, at least in part mediates regulation in *Bacillus subtilis*, our model organism, by phosphorylating seryl residue-46 in the paralogous phosphocarrier proteins of the PTS, HPr (the major co-repressor present in all low G+C Gram-positive bacteria) and Crh (the minor co-repressor present only in Bacilli). These phosphorylated PTS proteins interact directly with at least one well-characterized transcription factor, CcpA, to influence gene expression. We have discovered a second transcription factor, CcpB, which mediates catabolite repression under certain growth conditions and have shown that it exerts pleiotropic effects on gene expression. However, the mechanism of its action is largely unknown. Further, our transcriptome results showed that CcpA regulates many genes following patterns that cannot be explained on the basis of the established mechanism involving PtsK. We have cloned, sequenced and put into expression vectors all of the known genes encoding the constituents of these regulatory pathways. In the proposed research, we will combine transcriptome, bioinformatic, molecular genetic and biochemical approaches to establish the molecular mechanisms and physiological significance of CR and CA in *B. subtilis*. To identify targets and signal transduction pathways of CR and CA, we will (a) conduct whole transcriptome analyses of wild-type *B. subtilis* and a complete complement of isogenic mutants defective or altered for CR-mediating genes: *ccpA*, *ccpB*, *ptsK*, *ptsH1* and *crh*, (b) use bioinformatic approaches to define the DNA binding sites that promote CcpA and CcpB-mediated regulation, (c) use reporter gene fusions and molecular genetic approaches in vivo to confirm and extend the results obtained in (a) and (b), and (d) use in vitro biochemical approaches to establish the detailed molecular mechanisms of regulation. This research will reveal the molecular details and physiological consequences of protein kinase-mediated and protein kinase-independent CR and CA in low G+C Gram-positive prokaryotes, including a broad range of human pathogens.

Grant: 2R01GM057873-04
Program Director: SCHWAB, JOHN M.
Principal Investigator: TIUS, MARCUS A
Title: Cyclopentannelation in Total Synthesis
Institution: UNIVERSITY OF HAWAII AT MANOA HONOLULU, HI
Project Period: 1998/08/01-2005/07/31

DESCRIPTION: (provided by applicant) Brief enantioselective total syntheses of nakadomarin A and of guanacastepene have been proposed in which the asymmetric cyclopentannelation is the key step. Nakadomarin A, which has been isolated from an Okinawan Amphimedon sp. sponge in 1997 is biosynthetically related to the manzamines, but is structurally without precedent. It has a fairly complicated bridged system of six heterocyclic and carbocyclic rings. It is cytotoxic in the L1210 assay (IC₅₀ 1.3 microg/mL), shows inhibitory activity against cyclin dependent kinase 4 (IC₅₀ = 9.9 microg/mL), and has antibacterial and antifungal activity. Guanacastepene, which was isolated in 2000 from an endophytic fungus from a tree in Costa Rica, also has a unique carbon skeleton. It is active against both methicillin-resistant *S. aureus* (MRSA) and against vancomycin-resistant *Enterococcus faecalis* (VREF). The absolute stereochemistry of both compounds is in doubt, so the total synthesis will also constitute a proof of structure. Both natural products are structurally unique, therefore they may exert their respective activities through unusual mechanisms. Cancer and multi-drug-resistant human pathogens are two areas of concern in public health. In parallel with the total syntheses we will work to improve the methodology which was developed during the first funding cycle, and expand its scope. A long-range goal of the work is to determine whether the basic method can be adapted for the enantioselective synthesis of compounds with contiguous quaternary carbon atoms. This is still a difficult problem in synthesis.

Grant: 1R01GM059637-01A1
Program Director: SHAPIRO, BERT I.
Principal Investigator: NINFA, ALEXANDER J PHD
Title: Structure/Function Analysis of E. coli NRII
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2001/04/01-2005/03/31

DESCRIPTION: (Adapted from the Investigator's abstract): The NRI/NRII two-component regulatory system of Escherichia coli regulates the expression of genes involved in nitrogen assimilation in response to signals of carbon and nitrogen status. These signals regulate the activity of the PH and GlnK signal transduction proteins, which in turn regulate the kinase and phosphatase activities of NRII. The kinase and phosphatase activities of NRII control the phosphorylation state of NRI, which is only able to activate transcription when phosphorylated. We propose to study the mechanism and regulation of the kinase and phosphatase activities of NRH. These studies should advance our understanding of metabolic regulation in E. coli and the mechanisms of signal transduction by the two-component regulatory systems. Four main approaches are proposed. First, the structure of NRJI and domains derived from NRII will be investigated, along with the complex of P11 and NRII and the complex of PH with the isolated kinase domain of NRII. Second, the mechanisms responsible for the effect of mutations altering the kinase and phosphatase activities of NRII will be investigated. These studies will involve biochemical approaches using the isolated central domain of NRH and full-length mutant versions of NRII, as well as genetic studies of the regulation of the phosphatase activity. Third, studies with intact cells will be used to characterize the binding of PIT and GlnK to the kinase domain of NRII. Fourth, the epistasis relationships of mutations affecting the kinase and phosphatase activities of NRII will be investigated, and the ability of mutations affecting the phosphatase activity to complement one another will be investigated. Together, these approaches should result in a mechanistic understanding of the NRII kinase and phosphatase activities and their regulation by PH and GlnK.

Grant: 1R01GM059817-01A2
Program Director: CHIN, JEAN
Principal Investigator: VOGT, JANA L PHD
Title: REGULATION OF GLYCEROPHOSPHOINOSITOL TRANSPORT IN YEAST
Institution: CARNEGIE-MELLON UNIVERSITY PITTSBURGH, PA
Project Period: 2001/02/01-2001/11/30

DESCRIPTION (Applicant's abstract): The phospholipase-mediated deacylation of phosphatidylinositol (PI) to produce extracellular glycerophosphoinositol (GroPIs) is a major catabolic pathway in *S. cerevisiae* cells grown in inositol-containing medium. In times of inositol limitation, GroPIs is transported back into the cell in an energy requiring process mediated by the product of the GIT1 gene. This novel membrane permease has a number of interesting features that make it worthy of intensive study. Gitip is the first GroPIs transporter to be characterized in any organism, and there is evidence for the existence of such a transporter in higher eukaryotes. The GroPIs transported through Gitlp is used for the synthesis of PT and its inositol-containing derivatives, many of which play important roles in signal transduction. Thus, the activity of Gitip may impinge upon a number of important cellular events. In addition, evidence suggesting that the transport of GroPIs through Gitip is regulated by both phospholipid metabolism and phosphate metabolism raises the prospect of characterizing the regulatory networks linking these two fundamental aspects of cellular metabolism by means of the study of Gitip. Finally, the unique chromosomal location of GIT1 provides an opportunity to study the role of transcriptional silencing in regulating GIT1 expression. The Specific Aims of this proposal are to examine the regulation of GroPIs transport through Gitip at the transcriptional and posttranslational levels by 1) phospholipid metabolism, 2) the Unfolded Protein Response Pathway, 3) phosphate metabolism ,and 4) gene location.

Grant: 1R01GM059903-01A2
Program Director: PREUSCH, PETER C.
Principal Investigator: LEWIS, KIM A PHD
Title: NATURAL SUBSTRATES AND INHIBITORS OF MICROBIAL MDR PUMPS
Institution: TUFTS UNIVERSITY MEDFORD MEDFORD, MA
Project Period: 2001/03/01-2001/06/30

Our long-term objective is to understand the function and mechanism of microbial multidrug resistance pumps (MDRs). The main goal of this project is to identify natural substrates and inhibitors of microbial MDRs. According to our findings, berberine alkaloids are natural MDR substrates. Plants making berberine alkaloids also produce at least two MDR inhibitors (5'-methoxyhydnocarpin and pheophorbide) that act in synergy with berberine. We will use this model for an expanded search of new MDR substrates among plants producing antimicrobials, and for novel MDR inhibitors that are likely to accompany them. Our main focus will be on plants that make non-cationic antimicrobials that can be extruded by broad-specificity MDRs of Gram negative bacteria and yeast. This is a collaborative project that brings together the expertise of the PI in microbial multidrug resistance and the expertise of the co-PI in natural products chemistry. The overall project will flow from identifying antimicrobials that are MDR substrates, to finding MDR inhibitors in plants that produce these antimicrobials, to characterizing the interaction of these substances with microbial MDRs. The Specific Aims of this proposal are: 1. Identifying MDR substrates among plant antimicrobials. Plant antimicrobials will be screened with a panel of Gram positive bacteria, Gram negative bacteria and yeast in search of MDR substrates. We will screen a large collection of substances, representing the main types of antimicrobials (alkaloids, flavones, quinones, phenols) coming from a variety of unrelated plants. Antimicrobials that are more active against strains lacking MDRs are likely MDR substrates. 2. Searching for MDR inhibitors. Once MDR substrates are identified, the producing plant will be used to isolate MDR inhibitors. A bioassay-driven purification will be used, based on detecting inhibition of cell growth by a test extract in the presence of a sub-inhibitory concentration of an MDR substrate. The structure of newly identified MDR inhibitors will be determined. 3. Characterizing MDR inhibitors. (a) Spectrum of activity regarding the MDR family, and the type of organism will be determined. (b) Interaction of the inhibitor with MDRs will be studied using a transport assay with microbial cells. (c) SAR of MDR inhibitors will be performed. Relation to human health. MDR inhibitors are a new type of plant defense compounds that we are exploring. By potentiating other antimicrobials, MDR inhibitors may provide the key for developing plant antimicrobials into new antibiotics. MDR inhibitors will potentiate the action of conventional antibiotics, aiding eradication of multidrug resistant human pathogens.

Grant: 1R01GM060421-01A2
Program Director: MARINO, PAMELA
Principal Investigator: INOHARA, NAOHIRO PHD
Title: Nod1: An Apaf-like Activator of Apoptosis and NF-kB
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2001/07/01-2006/06/30

The immune response to microbial pathogens is initiated by recognition of specific pathogen components by host cells both at the cell surface and in the cytosol. While the response triggered by pathogen products at the surface of immune cells is well characterized, that initiated in the cytosol is poorly understood. Nod1 is a member of a growing family of proteins with structural homology to apoptosis regulators Apaf-1/Ced-4 and plant disease resistant R gene products. Nod1 promotes apoptosis when overexpressed in cells, but unlike Apaf-1, it induces NF-kappaB activation. NF-KappaB activation induced by Nod1 is mediated by the association of the CARD of Nod1 with the corresponding CARD of RICK, a protein kinase that activates NF-kappaB. Analyses with wild-type (wt) and mutant forms of both Nod1 and RICK have suggested that Nod1 and RICK act in the same pathway of NF-kappaB activation, where RICK functions as a downstream mediator of Nod1 signaling. Nod1 self-associates through its nucleotide-binding domain (NBD) and Nod1 oligomerization promotes proximity of RICK molecules and NF-kappaB activation. Like Nod1, intracellular plant R proteins contain N-terminal effector domains linked to a NBD and multiple leucine-rich repeats (LRRs) located C-terminally of the NBD. The LRRs of R proteins are highly diverse and appear to be involved in the recognition of a wide array of pathogen components. Remarkably, we find that bacterial lipopolysaccharides (LPS), but not other pathogen components, induced TLR4- and MyD88- independent NF-kappaB activation in human embryonic kidney 293T cells expressing trace amounts of Nod1. Like plant disease resistant R proteins, the LRRs of Nod1 were required for LPS-induced NF-kappaB activation. Furthermore, a LPS binding activity could be co-immunopurified with Nod1 from cytosolic extracts. Our hypothesis is that Nod1 is a mammalian counterparts of plant R gene products that may function as a cytosolic receptor for pathogen components derived from invading bacteria. We propose three Specific Aims to understand the biochemical mechanism by which bacterial LPS and Nod1 interact and to determine the role of Nod1 in the response to bacterial LPS in vivo.

Grant: 1R01GM060429-01A1
Program Director: RHOADES, MARCUS M.
Principal Investigator: KAJI, AKIRA PHD
BIOCHEMISTRY:BIOCHEM
RY-UNSPEC
Title: RIBOSOME RECYCLING FACTOR(RRF) IN PROKARYOTES
Institution: UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA
Project Period: 2001/01/01-2004/12/31

DESCRIPTION (Applicant's abstract): The long term objective of this project is to develop new antibacterial agents targeted against bacterial ribosome recycling factor (RRF) because RRF is an essential factor for prokaryotic translation but not for eukaryotic cytoplasmic protein synthesis. New antibacterial agents are urgently needed because of the emergence of resistant pathogens to the available antibiotics. RRF, together with elongation factor G (EF-G) or release factor 3 (RF3), catalyzes disassembly of the post-termination complex of ribosomes to recycle the ribosomes. In addition, RRF reduces translational error. RRF is a near perfect tRNA mimic. It is postulated that RRF binds to the ribosome and then translocated by EF-G on the ribosome in a fashion similar to tRNA. Inhibitors of tRNA binding to the ribosome and tRNA translocation will be used to examine this hypothesis. The interaction of RRF with ribosomes and EF-G will be studied by electron microscopy. Chemical cross-linking between RRF and the ribosome will be performed. Protection of ribosomal RNA from the effects of chemical agents by bound RRF will be examined to determine the ribosomal binding site of RRF. The possible influence of RRF on the ribosomal binding site of EF-G will also be examined. The gene encoding EF-G or RF3 will be mutagenized to explore the site of interaction with RRF. Other possible biological components which interact with RRF will be determined by identifying the intergenic suppressor genes of temperature sensitive RRF. The yeast two-hybrid system will also be utilized to find additional components that may interact with RRF. The active site of RRF will be explored using monoclonal antibodies against RRF. The mechanism of how RRF reduces translational error will be elucidated. RRF is postulated to release non-cognate or near cognate peptidyl tRNA from the ribosomal P (peptidyl)-site during the chain elongation steps. The release of cognate peptidyl tRNA from the ribosome by RRF and EF-G will be compared with that of the non- or near cognate peptidyl tRNA. At the A (acceptor) site, RRF is postulated to reduce binding of non- or near cognate aminoacyl tRNA by occupying the A site. The effect of RRF on the fidelity of aminoacyl tRNA binding will be tested. Two newly discovered inhibitors of the E. coli RRF reaction, purpurosmycin and T. maritima RRF, will be studied as to their mechanism of inhibition by biochemical as well as crystallographic means

Grant: 1R01GM061048-01A1
Program Director: LEWIS, CATHERINE D.
Principal Investigator: HINES, JENNIFER V BA
Title: Structure Function Studies of a RNA Antiterminator Bulge
Institution: OHIO UNIVERSITY ATHENS ATHENS, OH
Project Period: 2001/08/01-2006/07/31

DESCRIPTION (provided by applicant): Recently, a unique regulatory element in the transcription of tRNA synthetases in Gram-positive bacteria was identified. A novel RNA-RNA interaction occurs between uncharged tRNA and the mRNA 5' leader region of many Gram-positive tRNA synthetases. This interaction leads to antitermination of transcription and complete read-through of the gene. Without this interaction (i.e. in the presence of only charged tRNA), transcription termination occurs. The sequence and secondary structure dependence of this antitermination indicates a definite, sequence dependent interaction. However, based on these studies, it also appears as though the overall three-dimensional structure of the leader region and its complex with the uncharged tRNA plays a critical role in the antitermination function. The hypothesis is that there is a crucial tertiary structure/function correlation in the antiterminator bulge portion of the leader region. By studying structures of mutant sequences with decreased antitermination ability compared to the wild type, Dr. Hines can begin to construct a structure/function relationship. With further structural information, she can look at changes upon interaction with tRNA and begin to assay for and propose drug inhibitors. The long-range goal of this project is to disrupt the tRNA/mRNA interaction and function with small molecules that have been targeted to this system, using the structural information obtained in these studies. Such studies will lead to the development of novel antibiotics. Specific Aim I: Dr. Hines will investigate the solution structure of antiterminator bulge mutants where the mutation has been implicated by genetic studies to play a functional role. The structure of the mutants will be compared to the wild-type bulge in order to add to the knowledge of structure/function relationships for this system. Specific Aim II: Using either fully modified tRNA or a simplified tRNA acceptor stem model RNA she will investigate the solution behavior of the tRNA/antiterminator bulge interaction. She will investigate this interaction using native gels, fluorescence and NMR. Specific Aim III: She will determine tRNAIbulge sequence and structural requirements for functional interactions in vitro and antitermination in vivo. Specific Aim IV: She will begin to look at the effects small RNA binding ligands may have on the solution behavior of the antiterminator alone or complexed with tRNA acceptor stem.

Grant: 1R01GM061147-01A1
Program Director: SHAPIRO, BERT I.
Principal Investigator: FOSTER, JOHN W PHD
Title: MECHANISMS AND CONTROL OF ACID RESISTANCE IN E COLI
Institution: UNIVERSITY OF SOUTH ALABAMA MOBILE, AL
Project Period: 2001/03/01-2005/02/28

DESCRIPTION (Adapted from applicant's abstract): The gastric acid barrier presents a major obstacle to enteric pathogens and, as a result, is considered a key has strategy for preventing gastrointestinal infections. Yet some organisms, such as E. coli O157:H7, require extremely low oral infectious doses, suggesting the presence of extraordinary counter-defenses against acid stress. Our laboratory has discovered three highly effective and inducible acid resistance (AR) systems that protect pathogenic and commensal strains of Escherichia coli during passage through host gastric acid barriers. AR system 1 is glucose repressed, requires the alternative sigma factor 's and cAMP receptor protein for expression and will protect cells at pH 2.5 in minimal media. AR systems 2 and 3 depend upon amino acid decarboxylase/antiporter systems that utilize extra-cellular glutamate (AR system 2) and arginine (AR system 3) to protect cells during extreme acid challenges. However, the molecular basis of acid resistance provided by these three systems remains a mystery. The factors involved in system 1 AR are unknown and although some components of systems 2 and 3 have been identified, additional, unrecognized factors are clearly required. This application represents a comprehensive research plan employing biochemical and genetic approaches designed to probe the mechanisms and control of these critical acid survival systems. Focus is placed on systems 1 and 2 since systems 2 and 3 appear mechanistically similar. Specific aims are designed to examine the impact of AR systems 1 and 2 on pH homeostasis, characterize system-specific proton and counterion movements, probe the function of a critically important antiporter, identify requisite accessory proteins and define the complex transcriptional and post-transcriptional regulation of both systems. The molecular response of microbes to environmental stress is an exciting area of modern biology but often the functions of specific members are unknown and their roles in helping the cell survive the inducing stress obscure. The inducible acid resistance systems described provide a rare opportunity to learn how a microorganism senses a given stress (acid) and responds to that stress in a focused, purposeful manner to escape death. In a broader sense, knowledge gained from this study will also provide insights into fundamental questions concerning proton circulations, protein structure-function relationships and gene expression as each relates to acid stress

Grant: 1R01GM061606-01A1
Program Director: IKEDA, RICHARD A.
Principal Investigator: CECCHINI, GARY L
Title: Structure/Function of Complex II Oxidoreductases
Institution: NORTHERN CALIFORNIA INSTITUTE RES & SAN FRANCISCO, CA
EDUC
Project Period: 2001/07/01-2005/06/30

This project is intended to investigate the integrated events that occur during electron transport in membrane-bound respiratory Complex II. Two model systems are used, succinate- ubiquinone oxidoreductase (SQR, succinate dehydrogenase) and menaquinol-fumarate oxidoreductase (QFR, fumarate reductase) from *Escherichia coli*. Both are excellent model systems for investigating the function of Complex II that plays an important role in the metabolic processes that occur in mitochondria and is thus important for energy generation by the cell. These enzymes are structurally like their eukaryotic counterparts but much easier to manipulate genetically and for ease of production of large quantities of the proteins that can be studied by biochemical and biophysical methods. The enzyme complexes appear to have evolved from a common evolutionary precursor and are structurally and functionally very similar. Fumarate reductase usually functions in an anaerobic environment whereas, SQR a component of the Krebs cycle functions during aerobic metabolism. Functionally the enzymes can carry out the same reactions of oxidizing succinate to fumarate and reducing fumarate to succinate, however, QFR is much more efficient in both reactions than is SQR *in vivo*. These studies are intended to determine the structural and functional reasons for the differences in catalytic efficiency of the two enzymes. A recent high resolution structure of QFR allows design of specific site- directed mutations that will address the differences between the two enzymes around the catalytic site and covalent flavin cofactor. High resolution structures with inhibitors bound at the quinone binding sites will be obtained during these studies, as will the identity of amino acids involved in protonation/deprotonation with quinones. Additional studies will investigate how electron transport between the quinone and heme cofactors in QFR and SQR occurs. This research will help to understand the structure and function of quinone binding sites in respiratory complexes and the electron transfer reactions that take place at protein stabilized semiquinones in Complex II.

Grant: 1R01GM061629-01A1
Program Director: SCHWAB, JOHN M.
Principal Investigator: MOBASHERY, SHAHRIAR PHD
Title: Penicillin-Binding Proteins, Mechanism and Inhibition
Institution: WAYNE STATE UNIVERSITY DETROIT, MI
Project Period: 2001/04/01-2005/03/31

DESCRIPTION: (Applicant's Description) Penicillin-binding proteins (PBPs) are a group of enzymes involved in a number of functions in the assembly and regulation of bacterial cell wall. These enzymes are the targets of beta-lactam antibiotics for inhibition of bacterial growth. A multidisciplinary approach has been outlined for the study of PBPs, which builds on the mechanistic findings from this laboratory presented as Preliminary Results. Four Specific Aims are outlined. Specific Aim 1 details the plans for cloning, expression and large-scale production of two PBPs, one from *Escherichia coli* (a Gram-negative bacterium) and another from *Staphylococcus aureus* (a Gram-positive bacterium). These proteins will be used in the biochemical studies and also will be provided to Professor Judy Kelly of the University of Connecticut for crystallization. Specific Aim 2 describes our design and proposed syntheses for two cephalosporins that are incorporated with structural components of the cell wall (peptidoglycan). These cephalosporins, in conjunction with one that is already synthesized, are proposed as mechanistic probes for the transpeptidase reaction carried out by certain PBP in the last step of cell wall biosynthesis (cross-linking of cell wall). Biochemical and structural experiments are detailed for the use of these cephalosporins as probes of mechanisms for PBPs. An assay for the cell wall cross-linking reaction of the transpeptidases (a PBP) is described in Specific Aim 3. The enzymic reaction is biochemically dissected into the acylation and deacylation steps, for each of which a quantitative assay method is described. These methodologies will allow investigations of the mechanistic details of these PBPs. Furthermore, a series of four peptidoglycan derivatives have been proposed to investigate the requirements for a minimal substrate for the transpeptidation reaction of the PBPs. Specific Aim 4 details the search for novel non- β -lactam inhibitors for PBPs. These molecules will be synthesized and their potential PBP inhibitory and antibacterial activities will be investigated in both in vivo and in vitro experiments.

Grant: 1R01GM061689-01A1
Program Director: PREUSCH, PETER C.
Principal Investigator: MITRA, BHARATI PHD
Title: FUNCTIONAL ANALYSIS OF A LEAD/CADMIUM/ZINC ATPASE
Institution: WAYNE STATE UNIVERSITY DETROIT, MI
Project Period: 2001/06/01-2005/05/31

DESCRIPTION: (Applicant's Description) The superfamily of P-type ATPases catalyzes the energy-dependent transport of charged substrates across membranes. These ubiquitous pumps transport different cations and carry out a wide variety of functions, for example, they generate the transmembrane ion gradients necessary for nutrient uptake, signal transduction, and maintenance of suitable pH and ion concentrations as well as maintain the asymmetric distribution of phospholipids across plasma membranes of animal cells. Soft-metal P-type ATPases maintain homeostasis of the essential metals, Cu(I), Zn (II) and Co(II) and also mediate resistance to Ag(I), Pb(II), Cd(II), and other highly toxic metal cations. The human Cu(I)-transporting ATPases associated with Menkes and Wilson diseases, are examples of monovalent soft-metal ATPases whereas ZntA from *Escherichia coli*, the focus of this application, is a divalent soft-metal ATPase. The objective of this proposal is to use ZntA as a prototype to study the mechanism by which soft-metal ATPases recognize and transport specific metal ions. ZntA is ideal for this study because it has been cloned, overexpressed, solubilized and purified. An ATP-dependent transport assay has been developed for ZntA. A soft-metal dependent ATP hydrolysis assay has been optimized; ZntA is the first soft-metal ATPase for which this activity has been demonstrated. The specific aims include biochemical characterization of ZntA and analysis of the contributions of conserved residues and different domains, including the cysteine-rich amino-terminal domain of soft-metal ATPases, towards metal recognition and transport. The interaction between the metal-binding and transport domains with the ATP hydrolysis domain will be examined. The coordination and geometry adopted by different metal ions will be studied. Efforts to address these specific aims will include transport, ATP hydrolysis and phosphoenzyme formation assays, site-specific mutagenesis, and fluorescence EXAFS and x-ray structural studies.

Grant: 1R01GM061746-01A1
Program Director: SOMERS, SCOTT D.
Principal Investigator: JONES, STEPHEN B PHD
Title: Adrenergic Modulation Of Myelopoiesis In Burn & Sepsis
Institution: LOYOLA UNIVERSITY CHICAGO MAYWOOD, IL
Project Period: 2001/05/01-2005/04/30

DESCRIPTION Despite advances in critical care, patients with major burns remain at significant risk for septic complications. Previous work from our laboratory has shown that in the murine model of thermal injury and superimposed sepsis there is a bone marrow myeloid shift resulting in increased monocytopoiesis and arrest in granulocytopoiesis. Although sympathetic stimulation is known to accompany both clinical and experimental thermal injury and adrenergic stimulation modulates immune cell function, cytokine release and myelopoiesis under non-stressed conditions there is a lack of information on bone marrow norepinephrine (NE) release during trauma and its impact on myeloid commitment. Therefore, we hypothesize that burn injury and sepsis lead to increased bone marrow NE release which influences myeloid commitment and thus contributes to the subsequent cytokine dysregulation. We will test this premise in Specific Aim #1 by establishing that bone marrow sympathetic nerves are activated with thermal injury and sepsis leading to increased release of norepinephrine. Using pharmacologic, surgical and genetic models of sympathetic denervation we will establish that monocytopoiesis and granulocytopoiesis consequent to burn with infection are mediated by adrenergic stimulation in bone marrow. Specific Aim #2 will establish that bone marrow sympathetic stimulation with burn plus sepsis changes monocyte as well as monocyte progenitors to release enhanced amounts of cytokine in response to bacterial endotoxin. Using both tissue macrophage and those derived from bone marrow progenitors, cytokine responses to endotoxin as well as phagocytosis and chemotaxis will be determined in mice with denervated compared to neural intact bone marrow. Having established the role of sympathetic stimulation in mediating changes bone marrow myeloid commitment and function Specific Aim #3 will focus on the elucidation of cellular mechanisms by which NE mediates the observed changes. This aim will use paradigms involving bone marrow monocyte progenitor cells subjected to adrenergic agonists and antagonists to examine the proliferation and differentiation. Such findings will be extended using murine and human monocytic cell lines to investigate specific cell-signaling mechanisms. Results of the proposed research will provide important new information on the pathophysiology of thermal injury and sepsis and will lead to therapeutic approaches to enhance the survival of the critically injured.

Grant: 1R01GM061938-01A1
Program Director: DEATHERAGE, JAMES F.
Principal Investigator: WILSON-KUBALEK, ELIZABETH M BS
Title: Protein Crystallization of Lipid Tubule Substrates
Institution: SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA
Project Period: 2001/04/01-2006/03/31

DESCRIPTION (provide by applicant): Helical crystals of proteins are ideal specimens for structure determination by electron microscopy. A new method for facilitating the crystallization of proteins as helical arrays on lipid-tubule substrates has been developed. This strategy has come at a unique time where high-resolution (near atomic) structures are becoming available by electron crystallography and the number of identified proteins for which no structural data exists has exploded. This also coincides with advances in computing power, image processing and correction algorithms, and automation of electron microscopy that will allow rapid structural analysis of helical crystals of proteins. The long-term objective of this work is to rapidly obtain structural information about proteins and macromolecular complexes to better understand the biological functions. To meet this challenge, the proposed research revolves around experiments to broaden the general utility of lipid-tubule substrates for helical crystallization of proteins. Specifically, novel lipids that can serve to bind recombinant proteins through specific molecular interactions will be produced and tested. These novel lipids will be thoroughly characterized for their capacity to be incorporated into lipid-tubules, specifically bind proteins, and facilitate the formation of helical arrays of proteins on lipid-tubules. Another important component of the proposed research involves studies to define the practical limits and capacities of this new technique. To test the resolution limits of this approach, a three-dimensional (3-D) structure of a relatively small protein (~60 kD) will be determined that can be compared to an established atomic model of the same molecule. Also, the capacity of this method to reveal information about conformational changes in a large protein complex will be tested by obtaining 3-D structures of the complex in different nucleotide-associated states. It is envisioned that this work will ultimately expand the range of protein structures that can be determined.

Grant: 1R01GM061992-01A1
Program Director: SOMERS, SCOTT D.
Principal Investigator: ANGUS, DEREK C MD
Title: Genetic And Inflammatory Markers Of Sepsis
Institution: UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PA
PITTSBURGH
Project Period: 2001/04/01-2005/03/31

DESCRIPTION (Verbatim from the Applicant's Abstract): Sepsis, a diffuse inflammatory response to infection, occurs in over 450,000 cases per year in the US and frequently progresses to organ dysfunction and death. Although experimental studies using cells and animals have greatly improved our understanding of the pathophysiology of sepsis, there remains a remarkable paucity of biochemical and genetic data regarding the natural history of this important public health problem. In particular, there is a need for better markers of sepsis and outcome and a more rigorous evaluation of the complex relationships among the many genetic, inflammatory, and clinical factors that appear to influence the development and outcome of sepsis. Because pneumonia is the most common cause of sepsis, patients with this infection represent an excellent clinical model for studying sepsis in a relatively homogeneous population. We propose to study a large cohort of patients (n=2,703) with community-acquired pneumonia (CAP). Our study will be "piggy-backed" onto a multicenter trial of alternative hospital quality improvement initiatives that is already funded and slated to begin enrolling patient's early in 2001. In addition to collecting detailed clinical data, we will carry out careful genetic analyses, focusing on allelic variations in the coding or noncoding regions of genes whose products are important in the expression and/or regulation of the inflammatory response. We will also obtain measurements over time of the plasma concentrations or cell surface expression of several key inflammatory molecules. We will determine the influence of specific polymorphisms on the development, course and outcome of pneumonia and sepsis. We will test whether genetic predisposition to an exuberant inflammatory response protects against infection yet also increases risk for adverse systemic effects and outcome. We will compare genetic data from patients with results obtained from a cohort of healthy controls (n=300). We will test several existing hypotheses regarding the association of circulating inflammatory molecules with outcome. We will use time-varying regression analyses and probabilistic networks to explore in new detail relationships among genetic polymorphisms and the inflammatory response in sepsis. Finally, we will construct and evaluate two sets of clinical decision tools: i.) clinical risk prediction rules that incorporate genetic and inflammatory response variables with existing clinical factors, and; ii.) a state-transition simulation model of the course of sepsis that allows time-dependent estimates of the effects of alternative treatment decisions. This study will generate: new and valuable information regarding existing lines of inquiry and laboratory investigation; new hypotheses arising from the use of time-dependent modeling; and new clinical decision tools that have immediate and practical value for designing clinical trials and improving patient care.

Grant: 1R01GM062121-01
Program Director: JONES, WARREN
Principal Investigator: ERNST-FONBERG, MARY L MD
Title: FATTY ACYLATION OF INTERNAL RESIDUES OF A PROTEIN
Institution: EAST TENNESSEE STATE UNIVERSITY JOHNSON CITY, TN
Project Period: 2001/05/01-2005/04/30

Post-translational fatty acylation of internal protein residues is a powerful but poorly understood biological mechanism for altering a protein's behavior. It is important in diverse physiologic strategies, for example, signal transduction. The activation of Escherichia coli hemolysin (HlyA) toxin is used in this proposal as a model to study protein internal acylation. Pathogenic E.coli produce nontoxic proHlyA which is made toxic by the post-translational addition of 2 fatty acyl groups to internal residues, causing an extraordinary change in protein behavior. Acyl-acyl carrier protein (ACP) is the obligatory acyl-donor. HlyC is the internal protein acyltransferase, which forms an acyl-HlyC intermediate, evidence suggests an acyl-histidyl HlyC. Using a precise and direct assay, the reaction employing separately subcloned, purified proteins is a unique opportunity to explain the biochemical mechanism of an internal protein acyltransfer (the only one to be so studied) and its role in changing protein behavior. The following specific aims will be pursued with the goals of further understanding the biochemistry of protein internal residue acylation and associated changes in protein behavior and what defines an internal protein acylation site: 1. Site- directed mutation analysis, site-directed fluorescence, and chemical modification will be used to define the roles of several residues shown to be important in HlyC's catalytic function, and the acyl-HlyC intermediate will be characterized. 2. The reaction is likely the sum of 2 partial reactions, a ping pong kinetic mechanism; the reversibility of the first has been shown. Using HlyA labeled with selected radioactive fatty acyl groups, the reversibility of the second partial reaction will be shown. 3. Site-directed mutations of subcloned proHlyA, proHlyA- fragments containing either both or one or the other acylation sites, HlyA, and the respective acylated HlyA fragments will be studied to learn what defines an internal protein acylation site and what changes a protein undergoes upon acylation. Alterations in biological activity and fluorescence characteristics of site-directed fluoroprobes will be observed. E. coli hemolysin typifies one of a family of homologous, similarly activated, infectious protein toxins produced by diverse Gram negative bacteria. The toxins have remarkable organism and cellular specificities. The hemolysin scheme is an important recurring biological motif of rendering a protein toxic and secreting the infectious cellular-specific protein. Insight into the mechanism of this unique acyltransfer is of practical importance in providing a rationale for design of inhibitors of toxin activation as a potential therapeutic approach to severe infections with pathogenic E. coli where antibiotic therapy and subsequent toxin release worsen the clinical outcome.

Grant: 1R01GM062203-01
Program Director: IKEDA, RICHARD A.
Principal Investigator: ESCALANTE-SEMERENA, JORGE C PHD
Title: PROPIONATE CATABOLISM AND METABOLIC PATHWAY INTEGRATION
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 2001/01/01-2004/12/31

DESCRIPTION: (Adapted from the Investigator's abstract): An important and largely unanswered question in biology is how the cell integrates the large number of metabolic pathways that comprise its intermediary metabolism. The wealth of genomic information and new technologies developed in recent years make this exciting area of metabolism accessible to researchers. In the last four years, work in the PI's laboratory uncovered a pathway previously thought to be unique to eucaryotes for propionate catabolism in *S. enterica* and other procaryotes. Although substantial progress has been made toward a better understanding of the biochemistry of this pathway, gaps in our knowledge remain. Studies of regulation of expression of the genes encoding propionate-degrading enzymes identified an interaction between this pathway and the pathway for the degradation of 1,2-propanediol. The well-characterized genetic system of *S. enterica* offers a unique opportunity to dissect the molecular details of the metabolic integration of these two pathways. Interest in learning more about this example of metabolic pathway integration was increased by the involvement of a protein of *S. enterica* (CobB) for which there are orthologues in bacteria, archaea, and eucaryotes, including humans. In eucaryotes, CobB orthologues comprise the family of SIR2 regulatory proteins that are involved in the complex process of gene splicing. It has been demonstrated that the human SIR2 protein can compensate for the lack of the CobB protein during cell growth on propionate. The study of CobB function presents a worthwhile opportunity to learn about metabolic integration and other strategies that ensure proper cell function.

Grant: 1R01GM062205-01A1
Program Director: ANDERSON, JAMES J.
Principal Investigator: COLLADO-VIDES, JULIO PHD
Title: Gene Regulation E.coli Database Intergrated Environment
Institution: UNIVERSIDAD NACIONAL AUTONOMA DE CUERNAVACA,
MEXICO
Project Period: 2001/09/17-2004/08/31

DESCRIPTION (provided by applicant): The purpose of this application is to implement an integrated computational environment around a database on transcriptional regulation in E. coli. This database, RegulonDB, contains information gathered from the literature on regulatory elements and operon organization, their location in the genome, and experimental evidence supported by more than 1000 Medline original literature references. The project would transform the database into a useful tool for analysis of transcriptome and proteome experiments. Aim 1 is to gather data on growth conditions and their associated signal metabolites, and to expand the graphic capabilities of the system. Aim 2 consists in implementing and coupling the database with tools for genomic regulatory analyses, such as sequence retrieval, pattern discovery and pattern search, as well as a syntactic recognizer to detect multiple potential regulatory elements within an upstream region. Aim 3 centers on programs which would use as input a set of genes from a transcriptome experiment, and generate graphical or tabular information about their operon organization, upstream regulatory sites, functional classes of genes, and regulators affected. All these tools would integrate a flexible navigation path where the output of one query is the input for another one. Aim 4 consists in expanding and applying a Bayesian clustering method designed to deal with the heterogeneous type of information of gene regulation and metabolism. E. coli is here the model system, however, this approach and tools can in principle be applied to the study of other microbial organisms, resulting in more efficient ways to make use of currently available massive amounts of knowledge for the purpose of a better understanding of their biology, and potentially of their mechanisms affecting human health.

Grant: 1R01GM062206-01
Program Director: RHOADES, MARCUS M.
Principal Investigator: HUGHES, KELLY T PHD
Title: POST-TRANSCRIPTIONAL CONTROL OF FLAGELLAR FILAMENT
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2001/03/01-2005/02/28

DESCRIPTION: The development of the flagellar organelle in *Salmonella typhimurium* is a complex process in which the assembly pathway is intimately coupled to the regulation of gene expression and levels of subunit proteins to be assembled. The transcription of the flagellar filament protein is coupled to the completion of a basal structure, the hook-basal body. We have obtained evidence of post-transcriptional control of both the filament (FliC) and hook (FlgE) subunit proteins in response to flagellar assembly. The process of extracellular assembly is mediated by secretion through a mechanism commonly used to secrete pathogenicity proteins into the cytosol of eukaryotic host cells, the type III secretion system. We have evidence that the FlgE hook component of the bacterial flagella is regulated at the post-transcriptional level in response to the assembly of the cytoplasmic component of the flagellar-specific-type III secretion apparatus, the C-rod. We also have evidence that the intracellular level of FliC filament protein is regulated by a post-transcriptional mechanism in addition to the well-characterized transcriptional control. This research proposed here will determine the mechanism (i.e., trans-acting proteins and cis-acting mRNA signals) which mediate the regulation of FlgE and FliC levels in response to flagellar assembly. This research will also identify the cis- and trans-acting factors or signals that mediate the regulation of FlgE and FliC levels

Grant: 1R01GM062326-01A1
Program Director: CHIN, JEAN
Principal Investigator: BALL, JUDITH M PHD
Title: Transport and Lipid Interactions of a Novel Enterotoxin
Institution: TEXAS A&M UNIVERSITY SYSTEM COLLEGE STATION, TX
Project Period: 2001/06/01-2006/05/31

DESCRIPTION (provided by applicant): Rotaviruses cause severe, life-threatening diarrheal disease in young children resulting in over a million deaths worldwide. In 1996, we identified the first viral enterotoxin, rotavirus NSP4, and introduced a new mechanism of rotavirus-induced diarrhea. NSP4-induced diarrhea is mediated by a phosphoinositide signal transduction pathway that results in inositol triphosphate production, increased intracellular calcium, and chloride secretion. Yet, discrete lipid interactions and intracellular targeting of NSP4 in mammalian cells are unknown. Nor have structural studies been completed with defined model membranes. Our goals to define the intracellular transport and discrete cholesterol- and caveolin-1-interacting domains of NSP4 will be accomplished by combining innovative biophysical measurements, laser imaging, fluorescent spectroscopy and resonance energy transfer studies, with classical genetic and biochemical techniques. Our hypothesis is the enterotoxin-containing, cytoplasmic domain of NSP4 (cNSP4) is cleaved from the ER, transported to the cell surface in association with caveolin-1 and/or caveolar vesicles, and targeted to plasma membrane caveolae to interact with the signaling machinery of the cell. Our preliminary data show NSP4 and its active peptide, NSP4114-135, preferentially bind highly curved, anionic, cholesterol-rich membrane vesicles that mimic the plasma membrane microdomain, caveolae. Moreover, a cytoplasmic, C-terminal region of NSP4 is released from the ER when expressed in mammalian cells. We have shown cNSP4 colocalizes with caveolin-1, verifying that NSP4 and caveolin-1 are sorted to the same intracellular location. We now propose an in depth study of the mechanism of NSP4 transport in intestinal cells. The specific aims are to: 1. Characterize the intracellular location of the cleaved NSP4 fragment (cNSP4) and cNSP4-caveolin-1 interaction(s) in mammalian cells. 2. Determine the role of caveolin-1/caveolae in the intracellular transport of cNSP4. 3. Delineate the domains of NSP4 that influence cNSP4 transport in mammalian cells. This investigation will contribute new insights into our understanding of the newly discovered plasma membrane microdomains (such as caveolae); broaden our knowledge of intracellular protein-membrane/lipid interactions; contribute to our understanding of enterotoxin function; and disclose basic intracellular processes whereby other toxins may interact with the cell. Further, this study may reveal new intracellular protein transport pathways.

Grant: 1R01GM062344-01
Program Director: SOMERS, SCOTT D.
Principal Investigator: ALVERDY, JOHN C MD
Title: PSEUDOMONAS' EFFECTS ON THE GUT BARRIER FROM SURGERY
Institution: UNIVERSITY OF CHICAGO CHICAGO, IL
Project Period: 2001/02/01-2005/01/31

The mere presence of *Pseudomonas aeruginosa* in the intestine of critically ill surgical patients is associated with a 70% mortality rate--a 3 fold increase above matched patients who culture negative for this pathogen. We propose that within the intestinal tract of a surgically stressed host, physical and chemical environmental signals cause critical shifts in the virulence phenotype of *P. aeruginosa*. These effects result in a change in the behavior of intestinal *P. aeruginosa*, causing this bacteria, upon proper cue, to shift from an indolent colonizer to a life-threatening pathogen. In this proposal we provide strong evidence that a virulence determinant in *P. aeruginosa*, the PA-I lectin/adhesin, plays a key role in lethal gut- derived sepsis in a surgically stressed host. The hypotheses to be tested in this project are: 1) the PA-I lectin of *P. aeruginosa* is expressed in vivo in response to environmental cues in the intestinal tract including pH, redox state, and norepinephrine following surgical stress (hepatectomy) 2) the PA-I lectin of *P. aeruginosa* induces an epithelial permeability defect at the level of the intercellular tight junction resulting in paracellular transport of its lethal cytotoxins, and 3) the PA-I lectin of *P. aeruginosa* alters epithelial tight junctional permeability by activation of regulatory molecules involved in the expression of occludin, the rate limiting seal of the paracellular pathway. We will test these hypotheses using a novel mouse model of endogenous *P. aeruginosa* sepsis and cultured intestinal epithelial cells that we have extensively studied. Our specific aims to test these hypotheses are: 1) Determine the expression, location, and function of PA-I in *P. aeruginosa* harvested from different tissue sites in mice following surgical stress (hepatectomy) and cecal injection of live *P. aeruginosa* and following in vitro manipulation of the its physical microenvironment (pH, redox, osmolality, norepinephrine). 2) Determine the route of transport of the *P. aeruginosa* cytotoxins, exotoxin A and elastase, across cultured intestinal epithelial cells (Caco-2) in response to purified PA-I and selected mutants of live *P. aeruginosa*. 3) Explore potential cellular mechanisms of PA-I-induced decreases in intestinal epithelial barrier function. We propose that we should rethink our understanding of the gut theory of sepsis to include mechanisms by which pathogenic bacteria alter their virulence strategies in response to stressful changes in their local environment. Understanding the virulence determinants and cellular mechanisms that pathogens use to adhere to and modify the intestinal epithelial barrier may lead to therapies which can avoid nosocomial infection at a more proximate point in the care of the critically ill.

Grant: 1R01GM062362-01
Program Director: IKEDA, RICHARD A.
Principal Investigator: KURZ, LINDA C PHD
Title: MECHANISMS OF ENZYMES WHICH FORM CARBON CARBON BONDS
Institution: WASHINGTON UNIVERSITY ST. LOUIS, MO
Project Period: 2001/02/01-2005/01/31

DESCRIPTION: (Verbatim from the Applicant's Abstract) Our long-term goal is to understand the mechanisms of enzymes that catalyze carbon-carbon bond formation via Claisen condensations. Such enzymes are prominent in biosynthetic pathways (e.g., fats and cholesterol) and in energy-yielding pathways (e.g., tricarboxylic acid and glyoxylate cycles); we concentrate on citrate synthases. Their study will advance our general understanding of enzymatic catalysis. Citrate synthases are prototypes for several important catalytic strategies: carbonyl polarization to increase reactivity of the electrophilic substrate, facilitation of formation of the nucleophilic substrate carbanion, the use of unusual ionization states of histidine residues as acid catalysts, and changes in macromolecular conformation. We have four specific goals. 1. To complete determination of the detailed reaction profile using intramolecular substrate-isotope effects and transient kinetic methods (stopped-flow fluorescence and chemical quench). Oxygen exchange will be used to detect intermediates in the hydrolysis reaction. 2. To determine the structural basis of carbonyl polarization and carbanion stabilization. Structural and computational studies have implicated previously unstudied residues. We will change these residues to ones that cannot function in the ways propose and determine the consequences for carbonyl polarization (NMR and FTIR studies) and proton transfer (exchange studies). These solution results will be correlated with X-ray structures and calculations of the effects of mutants by our collaborators. 3. To determine the ionization states of the catalytic histidines in the various complexes of the enzyme. This exceeding important (but difficult) problem will be attacked using three different methodologies, solid-state NMR, solution-state NMR, and Raman spectroscopy. 4. To investigate the catalytic role of protein dynamics and flexibility by detailed mechanistic comparisons in a series of structurally homologous citrate synthases originating in organisms optimized to function at widely different temperatures. We shall correlate the stabilities of the intermediates and transition states with temperature to reveal which elementary steps in the mechanism are modulated by protein flexibility and dynamics. We will test predictions based on previous experimental and theoretical work.

Grant: 1R01GM062419-01
Program Director: HAGAN, ANN A.
Principal Investigator: GOLDEN, SUSAN S PHD GENETICS
Title: THE PATHWAY THAT SETS THE CYANOBACTERIAL CIRCADIAN CLOCK
Institution: TEXAS A&M UNIVERSITY SYSTEM COLLEGE STATION, TX
Project Period: 2001/03/01-2005/02/28

DESCRIPTION (Adapted from applicant's abstract): Cyanobacteria are the simplest organisms known in which to explore the mechanisms of circadian biological clocks. We have identified a gene, *cikA*, which is integral to relaying environmental information to reset the phase of the circadian clock in *Synechococcus* sp. strain PCC 7942, the model system for prokaryotic circadian rhythm studies. With the goal of elucidating the molecular basis of the clock and its entrainment to the photic environment, we will exploit this discovery by determining whether CikA acts directly as a photoreceptor and by identifying its signaling partners and their biochemical activities. CikA is a member of the phytochrome family of proteins, but lacks the expected cysteine residue that provides a ligand for bilin chromophore attachment in phytochromes and some other phytochrome-like proteins. The C-terminal domain has a well-conserved histidine protein kinase motif, and a segment of similarity with the receiver domains of response regulator proteins of bacteria. We will purify CikA directly from the cyanobacterium to determine whether a chromophore is attached, and if so, its chemical identity and influence on CikA function. This will be facilitated by modifying the *cikA* gene to add an affinity tag to the protein, and confirming that the modified gene is functional through complementation of a *cikA* null mutant. The role of autophosphorylation of the histidine protein kinase domain in resetting will be assessed. Genetic screens, both by a yeast two-hybrid assay and transposon mutagenesis in the cyanobacterium, will be used to identify proteins with which CikA interacts. The roles of these CikA partners in the phase resetting input pathway will be determined as well. In addition to providing fundamental insights into this most basic of biological processes, the project is also likely to uncover functional properties of currently cryptic phytochrome homologs that are present in the genomes of diverse prokaryotes.

Grant: 1R01GM062453-01
Program Director: SOMERS, SCOTT D.
Principal Investigator: BALAZY, MICHAEL PHD
Title: MASS SPECTROMETRIC IDENTIFICATION OF NEW LIPID MEDIATORS
Institution: NEW YORK MEDICAL COLLEGE VALHALLA, NY
Project Period: 2001/03/01-2006/02/28

DESCRIPTION (Applicant's abstract): The overall aim of this application is to characterize new mechanisms of biological membrane injury resulting from infection with bacterial endotoxin (LPS). The role of free radicals in LPS-induced endotoxemia is becoming well established, however, little is known about the processes involved in the damage to biomembrane lipids by nitrogen dioxide (NO₂) radical (a product of nitric oxide oxidation). We have developed a new methodology based on electrospray tandem mass spectrometry, which allows identification and quantification of specific lipid products formed by the reaction of NO₂ with arachidonic acid. Preliminary studies have revealed that this reaction generates a complex mixture of lipids containing characteristic products: trans isomers of arachidonic acid and lipids containing nitrogen-carbon bond (nitroeicosanoids). In addition, plasmalogen phospholipids reacted with NO₂, which resulted in complete removal of this group of lipids and generation of 1-lyso-phospholipids. We hypothesize that increased production of NO generates NO₂, which is a key radical that targets arachidonic acid and phospholipids, thereby causing membrane injury. Specific aims are to: 1) study relationships between the magnitude of trans-arachidonic acid generation and other markers of free radical damage (isoprostaglandin, nitrite/nitrate, nitrotyrosine); 2) examine the nitration of arachidonic acid and the effects of nitroeicosanoids on NO and cGMP levels in tissues; 3) characterize modifications of plasmalogen phospholipids in endotoxemia. In order to address the role of NO, L-NMA, a NO synthase inhibitor will be used to prevent generation of NO. Uric acid was shown to scavenge NO₂, and thus serve as a good probe to study effects of NO₂ in LPS-induced injury. Thus these probes will be used to determine the role of NO and NO₂ in arachidonic acid isomerization, nitration and plasmalogen degradation. We anticipate that in the long term the proposed studies will provide a foundation for a rational design of new strategies for development of new drugs (inhibitors of lipid isomerization and nitration), and therapies to treat symptoms of sepsis related to the NO₂-induced cytotoxicity.

Grant: 1R01GM062474-01A1
Program Director: SOMERS, SCOTT D.
Principal Investigator: VALLEJO, JESUS G MD
Title: Cardiac Depression in Gram-Positive Sepsis--Role of TLR2
Institution: BAYLOR COLLEGE OF MEDICINE HOUSTON, TX
Project Period: 2001/07/02-2006/06/30

DESCRIPTION (provided by applicant): The systemic inflammatory response induced by gram-positive bacteria is associated with considerable morbidity and mortality attributable to refractory hypotension, cardiac dysfunction and multiorgan failure. Despite the potentially important role that TNF- α , IL-1 β and NO may play in producing cardiac decompensation in human septic shock, little is known with regard to mechanism(s) by which bacterial pathogens induce their expression in the heart. A major advance in the understanding of the early events in gram-positive bacterial signaling has been the identification of Toll-like receptors (TLRs). Recent studies suggest that TLR2 may be a pattern recognition receptor that binds gram-positive bacteria and their cell wall components. In addition, TLR2 is an effective signaling molecule that activates NF- κ B, leading to cytokine production. The long-term objectives of this research initiative are not only to delineate the molecular pathogenesis of gram-positive septic shock, but also to develop strategies to prevent or attenuate the untoward effects of sepsis in the heart. Toward this end, the immediate specific objective of this application will be to delineate the role of TLR2 in the pathogenesis of myocardial dysfunction associated with gram-positive septic shock. Two closely interrelated hypotheses will be tested: first, signaling via TLR-2 is responsible, at least in part, for the induction of proinflammatory mediators associated with myocardial inflammation following infection with the gram-positive bacterium *Staphylococcus aureus*; second, TLR2 is responsible, at least in part, for the development of or *S. aureus*-induced left ventricular contractile dysfunction. These hypotheses will be tested in three Specific Aims. Specific Aim 1 will determine whether TLR2 mediates the inflammatory response induced by gram-positive bacteria in the heart. Specific Aim 2 will determine whether TLR2 mediates the cardiac response following infection with *S. aureus*. Specific Aim 3 will determine whether immunotherapeutic interventions designed to interdict signaling via TLR2 prevent and/or modify left ventricular dysfunction in gram-positive septic shock. These studies will provide definitive new information with respect to the mechanisms responsible for the deleterious effects of gram-positive sepsis on cardiac function and structure.

Grant: 1R01GM062508-01
Program Director: SOMERS, SCOTT D.
Principal Investigator: TRACEY, KEVIN J MD
Title: HMG-1 IN POST-OPERATIVE AND TRAUMATIC SEPSIS
Institution: NORTH SHORE UNIVERSITY HOSPITAL MANHASSET, NY
Project Period: 2001/02/01-2005/01/31

Mortality following surgery is caused primarily by complications of infection, but limited understanding of pathogenesis has hampered the development of new therapeutics. Endotoxin produced by all Gram- negative bacteria is lethal to mammals because it stimulates macrophages to release TNF and other potentially lethal factors. Maximal TNF levels are achieved within two hours after dosing with endotoxin; this timing is critical because even minimal delays in administering treatment against early mediators renders them ineffective. Paradoxically, endotoxin- poisoned animals succumb at latencies up to five days after the onset of endotoxemia, long after serum levels of the early cytokines TNF and IL-1 return to basal levels. Dr. Tracey and his colleagues recently identified a previously unrecognized "late" mediator of endotoxin lethality, a ubiquitous protein known as "high mobility group-1" (HMG-1) (Science, 1999, 285: 248-251). Endotoxin stimulates LPS-sensitive macrophages to release HMG-1; LPS-resistant macrophages from C3H/HeJ mice fail to release HMG-1. Serum HMG-1 levels are significantly increased from 16 to 32 hours after endotoxemia in mice. In serum from patients with surgical sepsis, high HMG-1 levels were associated with lethal outcome. Administration of rHMG-1 to mice is lethal, and delayed administration of anti-HMG-1 antibodies significantly protects against lethal endotoxemia, even when antibody administration is delayed until after peak TNF release. To date however, the effects of anti-HMG-1 or HMG- 1 itself in a clinically relevant animal model of trauma, injury, or hemorrhagic shock are unknown. Trauma and shock are known to induce a state of altered cytokine responsiveness in which macrophage activation states are deranged. It is theoretically possible that anti-HMG- 1 antibodies used in this setting would not improve survival; they may actually be deleterious. The objective of the studies outlined in this proposal is to determine whether HMG-1 mediates beneficial or injurious responses in the altered cytokine milieu of posttraumatic sepsis. The experiments proposed in the Specific Aims will define the effects of HMG-1 and antiHMG-1 antibodies in a clinically relevant model of sepsis in animals subjected to surgery and hemorrhagic shock. Studies of the binding and uptake of HMG-1 in human and murine monocytes, and the stimulation of cytokine release by HMG-1 itself, will yield important new information concerning the endogenous mechanisms underlying the systemic response to shock and sepsis.

Grant: 1R01GM062548-01
Program Director: FLICKER, PAULA F.
Principal Investigator: TANG, WEI-JEN PHD
Title: CALMODULIN REGULATION OF ANTHRAX AND ADENYLYL CYCLASES
Institution: UNIVERSITY OF CHICAGO CHICAGO, IL
Project Period: 2001/03/01-2005/02/28

Cyclic AMP (cAMP) and calcium ion are two key second messengers that transmit numerous extracellular and intracellular signals to control a plethora of physiological responses such as learning and memory, and control of heart rate. There is significant crosstalk between two signals transduced by these two second messengers. One of the intersection point involves calmodulin, a calcium sensor mediator that can activate two classes of adenylyl cyclase, the enzyme that synthesizes cAMP. One class is a toxin secreted from pathogenic bacteria such as edema factor from *Bacillus anthracis* and the second is adenylyl cyclase from higher eukaryotes such as mammalian type 1 enzyme (AC1). The long-term goal of this project is to elucidate the molecular mechanism that underlies the regulation of bacterial and mammalian adenylyl cyclases by calmodulin. Edema factor consists of two functional domains. The N-terminal portion (28 kDa) mediates association with protective antigen, a transporter produced by *B. anthracis* so that edema factor can be transported into eukaryotic cell. The C-terminal portion (60 kDa) of edema factor has high adenylyl cyclase activity (the turn over number is around 1,000 per sec) and the activity is highly dependent on calmodulin. We have expressed and purified the C-terminal catalytic domain of edema factor and have obtained diffracting crystals of edema factor alone and in complex with calmodulin. We propose to determine the molecular structures of both forms of the enzyme. We will then use these structures to generate a detailed catalytic model of edema factor activation. We will test this model with biochemical, spectroscopic, and additional crystallographic analyses. We will also use structure-based and genetic- based inhibitor screens to search for the high-affinity small molecules and peptides that block calmodulin activation and catalysis of edema factor. All mammalian membrane-bound adenylyl cyclases share a common structure, including two highly conserved domains (C1a and C2a) connected by the less conserved C1b and transmembrane domains. C1a and C2a form a soluble enzyme that can be activated by the alpha subunit of Gs. C1b region of AC1 consists of an amphipathic, alpha-helical region that is necessary for calmodulin activation. Mutational analysis suggests that activation of AC1 by calmodulin is distinctly different from that of edema factor. We propose to construct a calmodulin-sensitive soluble enzyme using C 1 and C2 domains of AC1 and its homologs. We will analyze calmodulin activates of the soluble AC1 in a manner similar to our analyses of edema factor. Success in this research will not only enhance our knowledge of how adenylyl cyclase is regulated, but also provide important structural insights into how calmodulin modulates the activities of its many other target proteins. In addition, success in finding a lead compound that inhibits edema factor would provide the means to develop better drugs to defend against the infection of *B. anthracis*.

Grant: 1R01GM062626-01A1
Program Director: ECKSTRAND, IRENE A.
Principal Investigator: WERNEGREN, JENNIFER J PHD
Title: MOLECULAR EVOLUTIONARY CONSEQUENCES OF ENDOSYMBIOSIS
Institution: MARINE BIOLOGICAL LABORATORY WOODS HOLE, MA
Project Period: 2001/08/01-2006/07/31

Genomes of most obligately endosymbiotic bacteria are characterized by fast evolutionary rates, extremely low GC contents, and small genome sizes. These shared features suggest that bacterial lifestyle severely affects patterns of molecular evolution. However, few comparative studies have addressed the processes that drive sequence and genome evolution in endosymbionts. The goal of the proposed study is to distinguish molecular and evolutionary mechanisms that shape endosymbiont genomes using phylogenetic and population genetic approaches. We propose to contrast rates and patterns of evolution across species of gamma-3 subdivision Proteobacteria, including free- living species of enterobacteria and related bacterial endosymbionts of carpenter ants, tsetse flies, and aphids. The specific aims of this study are (1) to quantify the impact of mutational biases, drift, and selection on protein evolution of intracellular bacteria, through molecular evolutionary and population genetic analyses of several protein-coding loci, (2) to explore forces that shape synonymous variation in endosymbionts, by testing for adaptive codon usage and quantifying mutational biases in these species, (3) to examine patterns of genome reduction in endosymbionts by testing for reduced genome size and repair gene loss in the uncharacterized endosymbionts of carpenter ants, and exploring mutational biases toward deletions in these small bacterial genomes. The long-range objective of this study is to understand the molecular evolutionary consequences of endosymbiosis in bacteria. We will approach this issue by examining genomic changes that occur in the context of obligate, intracellular associations. Results of this project will inform our understanding of the modes of evolution of intracellular bacteria, including both pathogens and mutualists, and will shed light on forces that shift the balance between mutation and selection in bacterial evolution. This work will also contribute to the development of more realistic evolutionary models for DNA sequences with biased base compositions, including genomes of organelles, and biased gene regions of vertebrates and other taxa.

Grant: 1R01GM062746-01
Program Director: RHOADES, MARCUS M.
Principal Investigator: SULLENGER, BRUCE A PHD
Title: DISRUPTING VIRAL GENES WITH GROUP II INTRONS
Institution: DUKE UNIVERSITY DURHAM, NC
Project Period: 2001/02/01-2005/01/31

DESCRIPTION: The overall goal of this proposal is to evaluate the ability of mobile group II introns to disrupt genes in mammalian cells focusing on HIV and CCR5 genes as targets. Group II intron mobility is mediated by a ribonucleoprotein (RNP) composed of an excised intron RNA lariat and an intron-encoded protein that has reverse transcriptase, DNA endonuclease and RNA splicing maturase activities. Mobility of the *Lactococcus lactis* group II intron occurs through a two-step process. First the intron catalyzes the reverse-splicing of itself into a DNA target site and then the intron RNA is reverse transcribed into DNA by the intron encoded protein. The insertion site on DNA is recognized by both the intron RNA, through about 14 base pairs, and the intron-encoded protein. Recently, we demonstrated that group II introns can be engineered to site specifically insert into targeted DNA sites in HIV- 1 pol and CCR5 genes by modifying the DNA binding sites present on the intron RNA. These retargeted introns retain the ability to insert into and disrupt their targeted DNA sites when they are transiently transfected into mammalian cells in complex with their intron-encoded protein. The specific aims of this proposal are: 1) To continue to explore the ability of group II introns to disrupt HTV and CCR5 genes via site specific DNA insertion following transfection of reconstituted group II RNPs into mammalian cells, 2) To evaluate group II intron splicing and mobility in mammalian cells following intracellular expression of the intron RNA and the intron-encoded protein, 3) To delineate the sequence requirements for *Lactococcus lactis* group II intron mobility in mammalian cells. 4) To evaluate the ability of group II RNPs to inactivate HIV proviruses by site-specifically inserting into viral DNA in infected cells and to render cells resistant to HIV infection and replication. These studies will also lay the foundation for development of mobile group II introns for targeted gene disruption in mammalian cells for therapeutic and research applications.

Grant: 1R01GM062940-01

Program Director: DEATHERAGE, JAMES F.

Principal Investigator: PARKINSON, JOHN S PHD
GENETICS:BIOCHEMICAL/M
LECULAR

Title: Aerotactic Signaling by the Escherichia coli Aer Protein

Institution: UNIVERSITY OF UTAH SALT LAKE CITY, UT

Project Period: 2001/07/01-2005/06/30

DESCRIPTION: (Provided by applicant): The Aer protein mediates aerotactic behavior in *Escherichia coli*. A growing list of other bacteria, including pathogens such as *Vibrio cholerae* and *Yersinia pestis*, have Aer homologs. The long-range goals of this project are to understand in molecular detail how the Aer protein senses changes in environmental oxygen levels and how that stimulus information is transmitted through the Aer molecule to control the cell's swimming movements. Aer appears to be located predominantly in the cytoplasm, but anchored to the inner face of the cytoplasmic membrane through a central segment of hydrophobic amino acids. To explore the role of membrane association in Aer function, the topology of the native molecule will be probed by accessibility to proteases and aqueous sulfhydryl modification reagents. Mutant proteins with deletions or substitutions in the hydrophobic segment will be tested for membrane association and aerotactic signaling ability in attempts to develop a soluble, active form of Aer. To identify conformational features that might play a role in Aer signal transduction, an extensive set of mutant proteins with single cysteine reporter residues will be constructed and used to examine intra- and intersubunit interactions between different regions of the native Aer molecule. Mutations that "lock" Aer into a stimulus-insensitive or stimulus-mimicked state will be isolated and used to trace the path of signal transmission through the molecule. Preliminary studies have established that the N-terminus of Aer binds flavin adenine dinucleotide (FAD), which might serve as a prosthetic group to monitor the redox state of an electron transport component. To test the redox-sensing model, in vitro assays of Aer signaling activity will be developed. The redox potential of FAD in native Aer will also be determined to identify electron transport components that could conceivably interact with Aer during stimulus detection. These studies promise to shed new light on biological mechanisms of oxygen-sensing. Moreover, the existence of Aer homologs in pathogenic bacteria suggests that aerotactic behavior might play a role in virulence. Thus, a molecular understanding of Aer signal transduction could lead to new anti-infection agents.

Grant: 1R01GM062987-01
Program Director: CHIN, JEAN
Principal Investigator: THANASSI, DAVID G PHD
Title: Mechanism of the Usher in Assembly and Secretion of Pili
Institution: STATE UNIVERSITY NEW YORK STONY STONY BROOK, NY
BROOK
Project Period: 2001/04/01-2006/03/31

DESCRIPTION (provided by the applicant): Pathogenic bacteria must assemble and secrete virulence factors in order to interact with their hosts and cause disease. Gram-negative bacteria have an outer membrane in addition to a cytoplasmic membrane and must secrete virulence factors across both these barriers. The mechanisms by which this occurs can be quite complex and are not well understood. The chaperone/usher pathway is a virulence protein secretion pathway that requires two components for secretion across the outer membrane: a periplasmic chaperone and an outer membrane protein termed an usher. The chaperone directs proper folding of the secreted proteins and prevents their engagement in non-productive interactions. The usher serves as an assembly platform at the outer membrane and provides a secretion channel to the cell surface. The chaperone/usher pathway is required for assembly and secretion of a superfamily of adhesive structures in a broad range of Gram-negative pathogens. The prototypical organelles assembled by this pathway are the P and type 1 pili expressed by uropathogenic *Escherichia coli*, the primary causative agent of urinary tract infections. P and type 1 pili are critical virulence factors, allowing binding and colonization of the kidney and bladder, respectively. The long-term goal of this proposal is to use pilus biogenesis by uropathogenic *E. coli* as a model system with which to understand virulence factor secretion in Gram-negative bacteria. More specifically, the structure and function of the usher will be investigated to elucidate the molecular mechanisms governing secretion across the outer membrane. The first specific aim is to create a detailed model of the structural arrangement of the usher in the outer membrane using computer analysis and epitope mapping techniques. The second specific aim is to probe function of the usher through generation and analysis of mutants. The third specific aim is to establish a cell-free system for pilus biogenesis based on reconstitution of the usher into liposomes. Such a system will provide an invaluable tool for studying the chaperone/usher pathway and analyzing mutants. The work described in this proposal will elucidate mechanisms of virulence factor secretion and create opportunities for the development of novel antimicrobial agents to treat not only urinary tract infections, but also a broad range of infectious diseases.

Grant: 1R01GM062994-01
Program Director: ECKSTRAND, IRENE A.
Principal Investigator: PERNA, NICOLE T BS
Title: Molecular Evolution of Microbial Pathogen Genomes
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 2001/05/01-2004/04/30

DESCRIPTION (Applicant's Abstract): We plan to develop, test and use computational tools for a fine scale investigation of the evolutionary history of microbial genomes. Understanding the evolutionary dynamics underlying the emergence of a pathogen with novel disease phenotypes will be greatly advanced by comparisons of the complete genome contents, organization and gene expression patterns of closely related organisms. We have designed an efficient and effective whole-genome pair-wise alignment tool and applied it to comparisons of the genomes of distinct pathogenic and non-pathogenic lineages of E. coli to delineate the genetic elements that distinguish among these strains. This preliminary work generated numerous insights into evolution of the E. coli genome in general and the relative roles of horizontal transfer and clonal divergence. We now propose further testing on a more diverse selection of pathogenic and non-pathogenic microorganisms to investigate the generality of what we have learned about evolution of microbial genomes and the robustness of our method. We also propose to expand this method to construct multiple alignments of all homologous regions of three or more genomes and to use phylogenetic analysis to partition the genome-scale multiple alignment into evolutionarily homogeneous units. Finally, we plan to develop an interactive Web-base viewer to integrate the results of these analyses with genome annotations and gene expression patterns. A whole-genome comparative approach to molecular evolution is the essential companion for an organismal level approach to studying the evolution of disease.

Grant: 1R01GM063004-01
Program Director: WEHRLE, JANNA P.
Principal Investigator: MATOUSCHEK, ANDREAS PHD
Title: Protein unfolding in a physiological system
Institution: NORTHWESTERN UNIVERSITY EVANSTON, IL
Project Period: 2001/04/01-2006/03/31

Description (provided by applicant): Regulated unfolding is critically important in the lifecycle of many proteins, such as those translocated across mitochondrial and chloroplast membranes, as well as those degraded by ATP-dependent proteases such as the proteasome. About half of all proteins synthesized in the eukaryotic cell are transported into or across a membrane. The protein translocation machineries are well defined biologically, but the means by which they transport and unfold proteins are not well understood at the biochemical and biophysical level. In contrast to protein folding, the mechanism of protein unfolding in the living cell has not been studied previously. The aims of this proposal are to understand the structural changes that occur in the unfolding protein prior to translocation and the molecular mechanisms of the unfolding machinery. The pathways of unfolding for a range of model proteins that translocate across membranes will be determined and compared with the pathway of spontaneous unfolding in solution. The mechanism of the unfoldase will be determined by inhibiting candidates either chemically or by mutation and measuring the effect on unfolding. The components of the import machinery that contribute to unfolding will be identified and the way in which they interact with each other the substrate protein determined. The hypothesis that the machinery unravels proteins by a physical pulling mechanism will be tested. This information is necessary to understand protein unfolding processes in the cell and to understand the function of a complex protein machine. The conclusions will also have broad implications for the understanding of protein translocation processes in the cell, in particular on the mechanisms that provide specificity to protein targeting to membranes. Interestingly, the unfolding processes during translocation and degradation share mechanistic features. Finally, the subject is directly relevant to human diseases. For example, an inherited form of oxalosis is due to the miss-sorting of an enzyme from peroxisomes to mitochondria. Since unfolding is not required for import into peroxisomes and the miss-sorted protein contains functional peroxisomal targeting information, preventing unfolding should correct the sorting defect.

Grant: 1R01GM063027-01
Program Director: WEHRLE, JANNA P.
Principal Investigator: ZUIDERWEG, ERIK R PHD
Title: Study of Allosteric Proteins by NMR
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2001/05/01-2005/04/30

DESCRIPTION (provided by applicant): The atomic-scale delineation of allosteric mechanisms has contributed much to the understanding of biomolecular function. Dr. Zuiderweg has obtained preliminary data that will allow us to study by nuclear magnetic resonance spectroscopy in solution (NMR), the allosteric mechanisms of Hsp70 proteins. NMR is capable of integrating the study of structure, dynamics and interactions and is therefore likely to contribute to the fundamental understanding of allostery, which thus far has been almost exclusively derived from comparisons of structures of proteins embedded in crystals. Dr. Zuiderweg has chosen the Hsp70 chaperone protein system as a target for his studies because its allosteric mechanism is currently unknown. The Hsp70's play a central role as the most abundant and most conserved systems aiding protein folding in vivo. Understanding of the functioning of these molecules is thus of relevance for the development of therapies for protein folding diseases. With newly developed NMR methods such as TROSY, spectral simplification by deuteration and specific labeling and the measurement of residual dipolar couplings, it is currently possible to study large proteins in solution at atomic resolution. As such, the study of allosteric proteins by NMR has come within reach; his target is 55 kDa. This first structural study of an allosterically functional Hsp70 protein will help delineate the conformational/dynamical changes that govern the allosteric coupling between nucleotide and substrate-binding domains. By NMR, it is possible to study these changes in solution, and monitor the effects on these parameters of adding different nucleotides, substrates, and co-factors such as phosphate, magnesium and potassium. In order to do so, Dr. Zuiderweg will first concentrate on the NMR description of the properties of 44 kDa nucleotide binding domains. In the next stage, Dr. Zuiderweg will move onward to the 55 kDa construct, and study its molecular parameters as a function of nucleotide and substrate binding combined. In order to facilitate this task, Dr. Zuiderweg will aim for the study of such a construct of the DnaK chaperone of the thermophilic bacterium *Thermus thermophilus*, which can be studied at elevated temperatures and hence gives rise to excellent NMR spectra.

Grant: 1R01GM063041-01
Program Director: SOMERS, SCOTT D.
Principal Investigator: MOLDAWER, LYLE L PHD EXPERIMENTAL
MEDICINE
Title: Targeted Interleukin-10 Gene Therapy in Sepsis Syndromes
Institution: UNIVERSITY OF FLORIDA GAINESVILLE, FL
Project Period: 2001/04/01-2005/03/31

DESCRIPTION (Verbatim from the applicant's abstract) The treatment of the hospitalized patient with sepsis remains a clinical conundrum, due in large part to the complexity of the host response to infection and tissue injury. Pro- and antiinflammatory cytokines play critical roles in the development of sepsis syndromes, but their production is often localized to individual tissues, rather than the systemic circulation. Current therapeutic approaches for the treatment of sepsis syndromes involve the systemic administration of biological response modifiers, such as cytokine antagonists or antiinflammatory agents. To date, none of these agents has proven effective, and one explanation may be that their systemic administration is an inefficient or even inappropriate means to treat a more localized inflammatory or immunosuppressive response. We have proposed that the targeted delivery of protein-based therapies using a recombinant adenoviral vector can directly modulate the inflammatory or apoptotic processes occurring in the target tissue, without systemic immune suppression or activation. Therefore, the overall objective of these studies is to determine the feasibility of adenovirus gene transfer as a therapeutic modality. More specifically, the goals of this proposal are to determine whether the forced overexpression of initially IL-10, but also in later years, a dominant negative I-KB super-repressor, will blunt the sepsis responses in target organs without producing systemic immune suppression. The two specific aims of the current application are: 1) To determine whether targeted expression of IL-10 in the lungs of mice with an adenoviral vector can prevent the development of respiratory distress syndrome in a zymosan-induced model of multisystem organ failure, without increased expression in other organs or the induction of T-cell hyporesponsiveness and immune suppression, and 2) To determine the mechanisms by which targeted expression of IL-10 in the thymus of mice can reduce caspase-3 dependent thymocyte apoptosis and improve outcome in a cecal ligation and puncture model of a compensatory antiinflammatory response syndrome. The studies proposed in this application will ultimately determine the utility and safety of adenovirus based gene therapy as a means of targeting protein-based therapies to individual tissues and organs in sepsis syndromes.

Grant: 1R01GM063205-01
Program Director: LEWIS, CATHERINE D.
Principal Investigator: GOLDMAN, YALE E
Title: Protein synthesis on single ribosomes
Institution: UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA
Project Period: 2001/07/01-2005/06/30

The research will test crucial functional aspects of mRNA-directed ribosomal protein biosynthesis at the level of individual ribosomes. Two new methodological developments, single molecule fluorescence polarization and force-feedback infrared laser optical trap will be applied, providing information currently unavailable about the mechanism of the ribosomal elongation cycle. Fluorescent probes will be inserted into elongation factors (EFs) and transfer RNA (tRNA) with known, predetermined orientations. The mechanism of proof-reading the genetic code and of translocation along the mRNA will be determined by investigating the kinetics, and angular relationships between, structural changes in EFs and tRNA. Whether EFs are motors or switches will be elucidated. The timing of tRNA motions through the ribosome will be compared to those of structural changes in the EFs. Using the optical trap, the relationship between mechanical tension in mRNA and velocity of peptide elongation (the force-velocity curve) of single ribosomes will be determined. The influence of altering substrate and product concentrations on the force velocity curve will be used in distinguishing hypothetical mechanisms of translocation. Protein synthesis is ubiquitous among living organism and the similarities between all ribosomes indicate that the elongation cycle is one of the most fundamental biological processes. Thus the collaboration proposed in this application between laboratories having expertise in motor protein and ribosome function, in vitro single molecule mechanics, and novel polarization spectroscopy will advance the understanding of ribosomal and G-protein function, and should impact very broadly in biophysics and biomedicine.

Grant: 1R01GM063259-01
Program Director: ECKSTRAND, IRENE A.
Principal Investigator: WIEDMANN, MARTIN PHD
Title: L. MONOCYTOGENES CLONAL GROUPS: ECOLOGY AND TRANSMISSION
Institution: CORNELL UNIVERSITY ITHACA ITHACA, NY
Project Period: 2001/03/01-2005/02/28

This project will combine the efforts of research groups at Cornell, the Technical University of Munich, and at the Wadsworth Center to quantify and statistically model associations between clonal *Listeria monocytogenes* groups and different environments and hosts. An independently funded parallel study in China will allow us to evaluate whether patterns and associations in the US are broadly applicable. Preliminary results show (i) that clonal *L. monocytogenes* groups differ in their likelihood to cause human and animal disease and (ii) that *L. monocytogenes* virulence genes appear to also be functionally important outside mammalian hosts (e.g., for interactions with protozoan cells). Laboratory studies will characterize phenotypes of clonal subgroups associated with specific environments or host species to determine the biological relevance of associations among clonal *L. monocytogenes* groups and different habitats. Laboratory studies will also define specific habitats (including non-mammalian host species) that may provide selective pressures for maintenance of virulence genes and the emergence of new *L. monocytogenes* strains. The outcome of this project will be a model of transmission dynamics of *Listeria* clonal groups and of environmental, host, and agent factors affecting transmission dynamics. Our studies will define environments likely to significantly and directly affect *Listeria* transmission dynamics when influenced by anthropogenic changes. The specific objectives of our study are: 1. Determine the distribution of clonal *L. monocytogenes* groups among (i) human hosts; (ii) non-primate mammalian hosts; and (iii) non-host related environments using culturing techniques and molecular and phenotypic approaches for characterization of isolates. 2. In parallel to Objective 1, use non-culturing-based techniques to determine the distribution of *L. monocytogenes* clonal groups in different environments using molecular approaches to avoid culturing bias. 3. Determine associations between *L. monocytogenes* clonal groups and different environments and host species and develop a transmission model for different clonal groups. 4. Determine the phenotypes of *L. monocytogenes* clonal groups associated with specific environments and hosts and determine the genetic basis for phenotypes associated with a preference for specific habitats.

Grant: 1R01GM063615-01
Program Director: ANDERSON, JAMES J.
Principal Investigator: HENKIN, TINA M
Title: REGULATION OF METHIONINE METABOLISM IN BACILLUS SUBTILIS
Institution: OHIO STATE UNIVERSITY COLUMBIUS, OH
Project Period: 2001/08/01-2005/07/31

DESCRIPTION (provided by applicant): A novel global regulation system for control of genes involved in methionine metabolism has been uncovered in *Bacillus subtilis*. Genes utilizing this mechanism, designated the S box family, contain in their mRNA leader regions a complex set of conserved primary sequence and structural elements, including a transcriptional terminator, competing antiterminator, and anti-antiterminator. Genetic analyses indicate that during growth in methionine, sequences in the leader are required for stabilization of the anti-antiterminator, which prevents formation of the antiterminator, which in turn allows termination. The molecular mechanism for control of the leader RNA structure in response to methionine levels is unknown, although preliminary studies suggest that binding of a regulatory factor is required to prevent readthrough. This system is widely used for control of methionine-related genes in a variety of Gram-positive bacteria, including important pathogens such as *Staphylococcus aureus*, and is also found in the Gram-negative bacteria *Chlorobium tepidum* and *Geobacter sulfurreducens*. Eleven transcriptional units are controlled by this mechanism in *B. subtilis* alone, so the total number of genes involved is high. The major goal of this study is to further investigate the molecular mechanism of transcription termination control, and to elucidate the physiological role of this system, using a combination of genetic and biochemical approaches. The required cis-acting sequence elements will be identified by site-directed mutagenesis. The trans-acting regulatory factors required for the methionine response will be identified, and the system will be examined both in vivo and in vitro. A requirement for ppGpp for efficient readthrough in vivo has been demonstrated, and the molecular basis for this requirement will be examined. Finally, the physiological role of genes of unknown function which appear to be regulated by this mechanism will be examined.

Grant: 1R01GM063618-01
Program Director: DEATHERAGE, JAMES F.
Principal Investigator: LEVITOV, LEONID S PHD
Title: Bacterial Aggregation & Pattern Formation
Institution: MASSACHUSETTS INSTITUTE OF TECHNOLOGY CAMBRIDGE, MA
Project Period: 2001/05/01-2005/04/30

DESCRIPTION (Provided by Applicant): Pattern formation is a key process in establishing the shape of a living organism, and it rests on intercellular communication. Patterning mechanisms remain poorly understood, primarily due to the complex organization of the eukaryotic systems in which they have been traditionally studied. Recently, it has been discovered that cells of *E. coli* and other gram-negative bacteria under certain conditions become sources of attractants. Chemotactic interactions in gradients of self-generated attractant produce different dynamically maintained multicellular structures, which, in turn, self-organize into spectacular spatial patterns. Chemotactic aggregation and self-organization in bacterial populations is a beautiful example of how an extremely regular and complex spatial pattern can be created even in the absence of a specialized genetic program encoding spatial information. Collective interactions by themselves--resulting from elementary processes such as excretion and degradation of a signal, motile behavior, and growth of individual bacteria--can encode nontrivial structure formation, more complex than could be anticipated from the behavior of a individual bacteria carrying out its own genetic program. We propose to study this phenomenon, both experimentally and by comparing to first principles theoretical analysis. Since the chemotactic behavior of individual bacteria has been quantitatively characterized for *E. coli*, a complete theory for the bacterial dynamics can be written down, with no free parameters. Discrepancies between the theory and experiments can thus be directly traced to uncertainties in the biochemistry. A principal goal of this research proposal is to use such models to rigorously find out how (local) microscopic rules of bacterial behavior and physiology translate into multicellularity and patterns on a (global) macroscopic scale. An example that we propose to analyze are slugs, large groups of *E. coli* which migrate and behave like a coherent, multicellular organism. The proposed work has two interconnected parts. Experiments include advanced imaging, standard methods of biochemistry and molecular genetics and will be aimed at investigating dynamics of essential elements of this phenomenon. In parallel, theoretical work (including both analytical methods and computation) will be conducted to construct models, which will both quantify and focus the experimental research.

Grant: 1R01GM063652-01
Program Director: EDMONDS, CHARLES G.
Principal Investigator: HU, JAMES C
Title: Protein Self-Assembly in Model Microorganisms
Institution: TEXAS A&M UNIVERSITY SYSTEM COLLEGE STATION, TX
Project Period: 2001/08/01-2005/07/31

DESCRIPTION (provided by applicant): With complete genome sequences available, it is now possible to examine all of the proteins in a genome for involvement in multisubunit assemblies. How different proteins are able to form stable complexes is of fundamental interest from the perspective of protein structure and folding. In addition, identifying proteins that physically interact can provide valuable clues about their biochemical and biological functions. Mapping domains within proteins that are responsible for oligomerization is an important part of structure-function analysis. This application describes experiments to simultaneously identify and localize oligomerization domains on a genome-wide scale. Genomic DNA fragments from *S. cerevisiae* that encode motifs that can self-assemble will be identified by a genetic approach based on gene fusion methods using *E. coli* as a host. Libraries of yeast DNA fragments cloned as gene fusions to the DNA binding domain of bacteriophage lambda cI repressor will be subjected to selection for repressor activity, which requires assembly into dimers or higher oligomers. Initial characterization of candidate motifs will exploit the unique ability of the repressor system to distinguish between dimers and higher oligomeric forms in vivo. While the selection and characterization of oligomerization domains from yeast is in progress, the search will be extended to find self-assembling domains from two bacteria, *E. coli* and *M. tuberculosis*, and two filamentous fungi, *N. crassa* and *A. fumigatus*. Although the primary focus of this proposal is on homotypic interactions, methods will be developed to use combinations of libraries in *E. coli*-based two-hybrid systems to examine protein motifs from *S. cerevisiae* that are sufficient to form heterotypic complexes. Oligomerization domains will be expressed and purified from *E. coli*. Size exclusion chromatography and analytical ultracentrifugation will be used to determine their oligomerization states. The boundaries of the domains that are necessary and sufficient to form stable complexes will be determined by partial proteolysis, followed by analysis of protease resistant fragments by N-terminal peptide sequencing and mass spectrometry. Structures of soluble oligomerization domains will be determined by X-ray crystallography. Expression vectors will be developed to use the oligomerization domains as "dominant negative" inhibitors in *S. cerevisiae* and in *E. coli*. This work will contribute to human health by providing important insights into protein taxonomy, materials for protein design, new tools for genetic studies in model organisms (*S. cerevisiae* and *E. coli*) and important human pathogens (*M. tuberculosis* and *A. fumigatus*), and new drug targets based on protein-protein interactions.

Grant: 1R01GM063716-01
Program Director: LEWIS, CATHERINE D.
Principal Investigator: XU, RUI-MING PHD
Title: STRUCTURE STUDIES OF TRANSCRIPTIONAL SILENCING
Institution: COLD SPRING HARBOR LABORATORY COLD SPRING HARBOR, NY
Project Period: 2001/08/01-2005/07/31

Transcriptional silencing is an epigenetic mechanism for control of gene expression. Genes located in certain regions of the eukaryotic chromosomes are permanently repressed. This heritable transcriptionally silent state is caused by altered chromatin structure that can be propagated from one generation to the next. Gene silencing has been observed in organisms ranging from yeast to human. Examples of silencing include mating-type loci in fission and budding yeasts, position effect variegation in *Drosophila*, and X-chromosome inactivation in mammals. In *Saccharomyces cerevisiae*, several protein complexes are known to be critical for establishing and maintaining transcriptional silencing at several genomic loci. They include the Silent Information Regulator proteins, Sir1p, Sir2p, Sir3p and Sir4p, and several sequence-specific DNA binding proteins, the Origin Recognition Complex (ORC), Rap1p and Abf1p. The structural basis of transcriptional silencing is still poorly understood. We will study the structure and function of the SIR proteins and ORC using X-ray crystallography as our principal method. The result of this study may facilitate an understanding of epigenetic inheritance, DNA repair and aging.

Grant: 1R01GM063786-01
Program Director: CHIN, JEAN
Principal Investigator: RYBENKOV, VALENTIN V PHD
Title: Mechanism of Bacterial SMC Complex MukBEF
Institution: UNIVERSITY OF OKLAHOMA NORMAN NORMAN, OK
Project Period: 2001/08/01-2006/07/31

DESCRIPTION (provided by applicant): Chromatin structure undergoes marked, tightly controlled changes during the cell cycle. Maintaining proper chromatin structure is essential for faithful execution of such fundamental events as chromosome replication, recombination, gene expression or cell division. Errors in either of the processes often result in genomic instability and chromosomal alterations potentially leading to carcinogenesis or developmental defects. However molecular mechanisms underlying chromatin dynamics remain largely unknown. The SMC protein family is an emerging group of cellular ATPases responsible for large-scale chromatin reorganizations in organisms ranging from bacteria to humans. These proteins have been implicated in such diverse range of cellular functions as chromosome cohesion and condensation, DNA repair and dosage compensation. It was found recently that the mechanism of 13S condensin, a *Xenopus* SMC complex responsible for the chromosome condensation during cell division, involves direct ATP-dependent deformation of the large-scale DNA structure. This is an entirely novel kind of enzymatic activity. This project focuses on further mechanistic characterization of the SMC proteins using MukBEF, a bacterial analog of 13S condensin, as a model protein. The specific aims of this project are: (1) characterize biochemical activities of MukBEF; (2) investigate cell cycle regulation of MukBEF; (3) investigate the architecture of MukBEF-DNA complex using electron microscopy; (4) investigate DNA deformation by MukBEF using single DNA manipulation technique. These studies are expected to yield a comprehensive scheme of the MukBEF-catalyzed reaction. These data will contribute to our understanding of the intracellular functions of MukBEF and will illuminate the role of SMC proteins in large scale chromatin dynamics.

Grant: 1R01GM063805-01
Program Director: FLICKER, PAULA F.
Principal Investigator: HOFF, WOUTER D PHD
Title: Partial Protein Unfolding as a Novel Signaling Mechanism
Institution: UNIVERSITY OF CHICAGO CHICAGO, IL
Project Period: 2001/07/01-2006/06/30

Receptor activation is at the basis of all biological signal transduction events. Malfunctioning of signaling pathways can cause cancer. We study the mechanism of receptor activation using photoactive yellow protein (PYP), a PAS domain photoreceptor, as a powerful model system. PYP exhibits rhodopsin-like photochemistry based on its p-coumaric acid (pCA) chromophore, and has been investigated by X-ray crystallography. We have recently shown that the presence of a protein crystal lattice greatly reduces the structural changes that occur during the formation of the pB photocycle intermediate, the presumed signaling state of PYP. In addition, our preliminary results strongly indicate that the pB intermediate is partially unfolded. In this proposal we will examine the role of partial protein unfolding upon receptor activation as a novel signal transduction mechanism. (i) We will establish the extent of partial protein unfolding during pB formation by NMR spectroscopy, and by two novel methods: light-induced H/D exchange and light-induced differential scanning calorimetry. In addition, we will examine the hypothesis that the pB intermediate is a molten globule state by studying changes in ANS fluorescence, changes in CD spectrum, and changes in the radius of gyration upon pB formation. (ii) We will examine the effects of the presence of a crystal lattice on the kinetics and thermodynamics of the photocycle using both wt- PYP and the E46Q mutant in both P6 3 and P6 5 crystals by flash photolysis at a range of temperatures. (iii) We will test the hypothesis that the Glu46-pCA couple constitutes the built-in default for the light-triggered protein quake in PYP. These experiments will reveal changes in functional dynamics imposed by a crystal lattice, and will determine the role of transient partial protein unfolding as a novel signal transduction mechanism that is anticipated to play a role not only in PYP but also in other signal transduction systems.

Grant: 1R01GM063853-01

Program Director: RHOADES, MARCUS M.

Principal Investigator: INOUE, MASAYORI PHD
GENETICS:BIOCHEMICAL/M
LECULAR

Title: Reverse Transcriptases in the Prokaryotes

Institution: UNIV OF MED/DENT NJ-R W JOHNSON MED PISCATAWAY, NJ
SCH

Project Period: 2001/07/01-2005/06/30

DESCRIPTION (provided by applicant): Contrary to the earlier belief, retroelements have also been found in bacteria. Retrons are such elements as these existing in some wild strains of *Escherichia coli* (<10 percent of the population), *Myxococcus xanthus* (all natural isolates), and *Vibrio cholerae* (only in pathogenic strains). They encode reverse transcriptase (RT) evolutionarily related to retroviral RTs and other eukaryotic RTs. Bacterial RTs are responsible for the synthesis of a peculiar satellite single-stranded DNA called msDNA (multicopy single-stranded DNA), in which a single strand DNA (cDNA) is branched out from an internal rG residue of a highly structured RNA molecule forming a unique 2',5'-phosphodiester linkage. The outstanding mysteries concerning msDNA synthesis are how individual bacterial RTs highly diverse each other are able to recognize their cognate RNA molecule, and how cDNA synthesis is primed from a specific internal G residue (branching G residue) in the single RNA molecule. We recently demonstrated that the 91-residue C-terminal domain of RT-Ec86 is responsible for the specific recognition of a stem-loop structure unique to the primer-template RNA molecule for RT-Ec86. Notably, this recognition stem-loop structure locates downstream of the branching G residue used for the cDNA priming reaction. On the basis of our recent results, we hypothesize that the seemingly primitive bacterial RTs retain the unique ability to utilize the C-terminal thumb domain region to specifically recognize their cognate stem-loop structure downstream of the branching G residue. This unique RNA-thumb domain interaction allows the 2'-OH group of the branching G residue to be correctly positioned at the active site of RTs to which the first nucleotide complementary to the template RNA is added forming a 2', 5'-phosphodiester linkage. In this proposal, we first attempt to construct molecular models of RT-Ec86 and its complexes with the primer-template RNA and the final msDNA product. On the basis of these models, we will take experimental approaches to determine the precise molecular mechanisms for the interaction between the thumb domain and the recognition stem-loop structure, for the cDNA priming reaction from the 2'-OH group of the branching G residue, and for the cDNA elongation reaction leading to the final msDNA product.

Grant: 1R01GM063862-01
Program Director: CHIN, JEAN
Principal Investigator: DALBEY, ROSS E
Title: Inner Membrane Protein Assembly in Bacteria
Institution: OHIO STATE UNIVERSITY COLUMBUS, OH
Project Period: 2001/06/01-2005/05/31

Protein export is fundamental to many cellular processes including secretion, organellar biogenesis, and signaling. Since hundreds to thousands of proteins must cross at least one membrane before arriving at their final destination, protein translocation is a basic process in cell biology. In prokaryotes and eukaryotes, most of the components required for efficient assembly and export of proteins into and across biological membranes are well known. In bacteria, the insertion of the majority of membrane proteins is catalyzed by a complex of proteins (SecYEG) that receives membrane protein precursors and ensures their proper distribution within the bilayer. However, there is a class of membrane proteins whose assembly occurs independently of the Sec complex. These Sec-independent membrane proteins were thought to assemble into membranes directly without the assistance of a protein complex. Recently, we discovered that the assembly of Sec-independent integral membrane proteins into the inner membrane of *Escherichia coli* is dependent on a new accessory protein coded by the *yidC* gene. YidC is a medium sized (60 kDa) integral membrane protein. Null mutations in YidC are lethal, indicating that YidC is essential for cell growth. Homologs of YidC have also been identified in mitochondria and chloroplasts. Depletion of YidC from growing cells inhibits membrane insertion of Sec-independent proteins. In addition, the assembly of Sec-dependent membrane proteins is significantly delayed, while there is no effect on the export of secreted proteins. In order to understand the role of YidC in membrane protein assembly we plan to pursue the following specific aims: (1) Examine the global role of YidC in membrane protein assembly; (2) Characterize the components involved in the YidC-dependent membrane assembly pathway; (3) Reconstitute YidC-dependent membrane assembly using purified components; and (4) Determine the sites of interaction between YidC and its substrates. These studies will help to understand this novel bacterial membrane protein assembly pathway and perhaps help to explain similar events in eukaryotic cells.

Grant: 1R01GM063919-01
Program Director: CHIN, JEAN
Principal Investigator: BOWIE, JAMES U PHD
Title: Membrane Protein Stability
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 2001/08/01-2005/07/31

Although a quarter of all genes encode membrane proteins and the vast majority of therapeutic drugs target membrane proteins, remarkably little is known about membrane protein structure and function. This knowledge gap has occurred largely because often poor behavior of membrane proteins in detergent solution that leads to many intractable technical problems. We recently discovered that single point mutations in a marginally stable membrane protein, diacylglycerol kinase, can dramatically improve its stability in detergent. Moreover, stabilizing mutations are not rare. The goal of the work in this proposal is to learn more about how mutations stabilize membrane proteins in detergent. This work could lead to both practical improvements in our ability to handle membrane proteins and also provide fundamental insight into membrane protein structure. Aim I. Identify and characterize additional stabilizing mutations in diacylglycerol kinase and bacteriorhodopsin. This aim will not only provide fodder for subsequent aims, but by identifying a large number of mutations, it may be possible to develop predictive rules regarding side-chain types and sequence positions that are likely to stabilize. Aim II. Solve structures of stable mutants. We will learn in atomic detail how many of the mutants stabilize, providing new insights into how membrane protein structures are held together. Aim III. Examine the mechanism of inactivation in detergent solution for diacylglycerol kinase, and bacteriorhodopsin. This work will lead to a better understanding of why certain mutations stabilize and may also aid future detergent improvements by highlighting weaknesses in existing detergents. Aim IV. Develop a general screen for stabilizing mutations. We have devised a strategy for stability mutant screening that does not depend on a rapid activity assay. This method could bring the benefits of high stability to essentially any membrane protein of interest.

Grant: 2R15GM055945-02
Program Director: RHOADES, MARCUS M.
Principal Investigator: GUPTA, RAMESH PHD
Title: RNA Splicing in Archaea
Institution: SOUTHERN ILLINOIS UNIVERSITY CARBONDALE, IL
CARBONDALE
Project Period: 1998/05/01-2005/04/30

Post-transcriptional RNA processing can regulate gene expression, which is essential for the control of cellular metabolism, growth, and differentiation. Broad, long-term objectives of this AREA application are to characterize various RNA processing events in archaea. Both archaea and bacteria are prokaryotes; yet archaea exhibit several molecular features resembling eukaryotes. This proposal specifically deals with pre- tRNA splicing in *Haloferax volcanii*, a halophilic archaeon. A long-term goal is to also clone and overexpress the gene for *H. volcanii* splicing ligase. The specific aims of this proposal are: Purification of *H. volcanii* tRNA splicing ligase; Determination of the structural requirements for the substrates of *H. volcanii* splicing ligase; Characterization of *H. volcanii* splicing ligase reaction; Determination of the presence or absence of 2'- O-methylcytidine (Cm) modification at the wobble position of the tRNA produced in vivo in *H. volcanii* by an intronless tRNA gene; and, Determination of the effect of Cm modification at the wobble position of tRNA, on the accuracy of translation in *H. volcanii*. *H. volcanii* splicing ligase is inactivated under low salt concentrations containing solutions. Therefore, the ligase will be purified by the methods where high concentrations of sodium/potassium salts are maintained continuously. Certain procedures where ammonium sulfate or organic solutes replace these salts, will be used for the purification. Several modified in vitro produced and commercially available substrates will be tested in ligation reactions for determining the structural requirements for the substrates of ligase. Ligase-substrate binding will be characterized by gel-shift assays using some of these substrates. Various different substances and modified substrates will be examined for their effect on the ligase reaction. Role of the intron in modification at the wobble position of tRNA and its effect on the accuracy of translation will be tested by genetic methods, using a specifically modified reporter protein system and an intron-deleted tRNA gene. The tRNA product of this intronless gene will be characterized for the presence or absence of Cm modification at the wobble position of the tRNA, by RNase T1 fingerprinting.

Grant: 1R15GM062197-01
Program Director: MARINO, PAMELA
Principal Investigator: SLOAN, GARY L PHD
Title: ZOOCIN A AND ITS ASSOCIATED IMMUNITY FACTOR
Institution: UNIVERSITY OF ALABAMA IN TUSCALOOSA TUSCALOOSA, AL
Project Period: 2001/04/01-2005/03/31

DESCRIPTION (Verbatim from Applicant's Abstract): The long-term goal of this research is to understand the control and transfer of information that allows some bacteria to produce toxic products to which they are not intrinsically resistant. The model systems being studied are lysostaphin, a plasmid-encoded staphylolytic glycyglycine endopeptidase produced by *Staphylococcus simulans* biovar *staphylolyticus*, which hydrolyzes the polyglycine cross bridges in the cell wall peptidoglycans of other staphylococci, and zoocin A, a chromosomally encoded streptococcolytic enzyme of unknown mechanism of action that is produced by *Streptococcus equi* subsp. *zooepidemicus* 4881. A comparison of the sequences of the genes for lysostaphin endopeptidase (*end*) and zoocin A (*zooA*) has revealed a high degree of similarity between these two enzymes; however, our preliminary investigations have revealed that zoocin A is most likely not simply an endopeptidase that hydrolyzes peptidoglycan cross bridges in susceptible streptococci. Both organisms carry resistance genes that also have a high degree of similarity (designated *epr*, for lysostaphin endopeptidase resistance and *zif*, for zoocin A immunity factor) immediately adjacent to the enzyme genes and oriented in the opposite direction to them. Both *epr* and *zif* have a high degree of similarity to *femAB* (factor essential for methicillin resistance) in staphylococci. The gene products for *epr* and *femAB* are known to be involved in the synthesis of cross bridges in the peptidoglycans of staphylococci; *Epr* specifies for the insertion of serines in these peptides whereas *FemA* and *FemB* specify for the insertion of glycines. Even though *Zif* is very similar to *Epr*, *FemA*, and *FemB*, our preliminary investigations have revealed that it is not involved in the biosynthesis of cross bridges. In addition, both *end/epr* and *zooA/zif* are bracketed by what appear to be transposable elements, suggesting that there may have been a horizontal transfer of DNA fragments containing these genes at some point in time. The specific aims of the proposed project are 1) to identify the site of action of zoocin A on the peptidoglycans of susceptible streptococci; 2) to identify the precise change made in streptococcal cell wall peptidoglycans by *Zif* and the cellular location of this protein; and 3) to determine if *end/epr* or *zooA/zif* can be transferred to other gram-positive organisms by natural mechanisms for genetic transfer.

Grant: 1R15GM062199-01
Program Director: ANDERSON, JAMES J.
Principal Investigator: CURRAN, JAMES F PHD
Title: FRAMESHIFT REGULATION OF E COLI RNA POLYMERASE GENES
Institution: WAKE FOREST UNIVERSITY WINSTON-SALEM, NC
Project Period: 2001/06/01-2004/05/31

DESCRIPTION: Proposed is a study of what may be a novel set of properties for the E. coli RNA polymerase core protein genes (rpoA, B and C genes). These genes are known to be growth rate regulated, and that regulation occurs by several transcriptional and translational mechanisms, but the detailed mechanisms and their coordination are largely obscure. Our sequence analyses show that the 5'ends of these genes contain UUU-Ynn dicodons, which are known to be highly frameshift-prone, and which are strongly avoided in other highly expressed genes. That these UUU-Ynn sequences occur near the 5'ends of all three rpo genes, and that this pattern is shared by diverse bacteria, suggests that these sites may have undiscovered, important functions. We will determine whether these sequences are frameshift-prone and whether frameshifting can contribute to regulation of these genes. The rpoB gene may be especially interesting because it encodes a potential frameshift-polypeptide that would be large enough to have function. This feature is also conserved among diverse bacteria. We will determine whether expression of this polypeptide has any global effect on cell physiology, and whether it specifically affects rpo gene expression. These studies may uncover interesting phenomena in the regulation and expression of the physiologically important rpo genes. This work will also train undergraduates interested in biomedical careers.

Grant: 1R15GM062792-01
Program Director: ANDERSON, JAMES J.
Principal Investigator: WHITE-ZIEGLER, CHRISTINE A BA
Title: Rim J and thermoregulation of pili transcription
Institution: SMITH COLLEGE NORTHAMPTON, MA
Project Period: 2001/06/01-2005/05/31

DESCRIPTION: (Adapted from the Investigator's abstract): The overall goal of the study proposed here is to characterize RimJ and understand how RimJ controls transcription of the pyelonephritis-associated pili (pap) operon in response to changes in temperature. Expression of Pap facilitates attachment of uropathogenic *Escherichia coli* to epithelial cells and colonization of the upper urinary tract, leading to infections. Expression of the pap operon is controlled by temperature such that transcription occurs at 37 C, but not at lower temperatures (22-26 C). Mutations in rimJ lead to a loss of this thermoregulatory response such that transcription of the pap operon occurs at the nonpermissive temperature. RimJ is a N-terminal acetylase that modifies the ribosomal protein S5. In this study, the function of RimJ will be more fully characterized by obtaining additional mutations within rimJ. These mutants will be characterized for their effect on pap thermoregulation, RimJ stability, and acetylation activity. Along with the mutational study, DNA sequence analysis will be used to compare RimJ to other N-terminal acetylases to determine areas of conservation that may delineate important functional domains of this enzyme. Two dimensional gel electrophoresis will be used to determine if S5 is the only substrate of RimJ or if it has another target that is important for pap thermoregulation. In addition, genetic techniques will be utilized to assess whether RimJ represses transcription in response to other environmental stimuli or if it controls other fimbrial operons besides pap, thus demonstrating whether RimJ is a more global regulator. These studies should provide illumination about the mechanism by which bacteria sense temperature and subsequently control gene expression. They will also shed light on the role of N-terminal acetylation, a process that is not well studied in prokaryotes. These studies are important clinically because there are several fimbrial operons that share regulatory mechanisms with pap that may also be controlled by RimJ. An understanding of how RimJ represses transcription is important as we look towards anti-infective strategies where signal transduction pathways can be targeted to prevent the expression of virulence determinants important for colonization.

Grant: 1R15GM063613-01
Program Director: CHIN, JEAN
Principal Investigator: FIRSHEIN, WILLIAM PHD
Title: Host-Plasmid Initiators of Membrane DNA Replication
Institution: WESLEYAN UNIVERSITY MIDDLETOWN, CT
Project Period: 2001/09/01-2004/08/31

DESCRIPTION (provided by applicant): Many key metabolic reactions are controlled or influenced by components that are associated with the cell membrane of prokaryotes. A vital reaction, which has emerged as subject to such control, is DNA replication. Of many reasons for this control, the lack of a nucleus in prokaryotes has required the genetic material to seek a cellular site where important events involved in its replication and segregation into a daughter cell could be sequestered. A great deal of evidence has been presented to support this supposition and the replicon model of Jacob et AL (1963, Cold Spring Harbor Symp. Quant. Biol. 28:329-348) still represents a good conceptual framework (with modifications) to explain how such replication and segregation are linked by the cell membrane. The proposed investigations will continue to focus on a remarkable pair of initiation proteins (TrfA) encoded by overlapping genes from a medically important broad host range plasmid RK2 cultured in its E. coli host. These proteins (33 and 43 kDa) express what appears to be a fundamental paradox, that of interacting in a "soluble" environment (namely the cytoplasm) with the origin region of plasmid replication (ORIV) while being sequestered in a hydrophobic environment (namely the membrane) so as to anchor them to a stable site. A critical region near the carboxyl terminus of both proteins consisting of hydrophobic amino acids (HR) was identified as an important domain for membrane interaction and plasmid viability. Other regions, however, are also involved in membrane binding, although there is no obvious amphipathic helix or hydrophobicity for them. One such region is not only associated with the amino terminal portion of the 33 kDa species but also has a synthetic lethality for the host cell indicating an interaction with a host component. These results will be extended in two major directions. 1) an attempt to detect an interaction of the TrfA proteins with a host component by multicopy suppressor analysis and 2) demonstrate that a specific submembrane domain of the E. coli host represents the actual site for plasmid DNA replication and the embodiment of the replicon model for prokaryotic DNA replication.

Grant: 1R15GM063637-01
Program Director: ANDERSON, JAMES J.
Principal Investigator: KUTTER, ELIZABETH M PHD
BIOPHYSICS:BIOPHYSICS-
UNSPEC
Title: T-Even Phages for Food-Chain Biocontrol of e.coli 0157
Institution: EVERGREEN STATE COLLEGE OLYMPIA, WA
Project Period: 2001/08/01-2003/07/31

The long-term objectives of this proposal are (1) to investigate the dynamics and physiology of T-even phage replication under conditions relevant to the natural environment and to potential therapeutic applications, i. e. during anaerobic respirative and fermentative growth of the host, (2) to identify and characterize T-even phages capable of infecting E. coli 0157:147 anaerobically and use appropriately-designed mixed-culture fermenters to assess the potential for use of these phages as a means of reducing the 0157:147 load in the guts of cattle, and (3) to identify regimes for efficiently producing and effectively administering the chosen phages. Preliminary experiments have identified 12 T-even phages that can infect 0157 and have shown that some T-even phages can infect E. coli growing under conditions of anaerobic respiration and/or fermentation, but the process is substantially different than during aerobic infection, and also differs between fermentation and respiration, between different host strains and between different related phages. Studies will include effects of available nutrients, electron acceptors, and pH and will determine various phage growth parameters -- adsorption efficiency, lysis time and lysis inhibition patterns, intracellular phage production, and burst size. This data will be used to set up continuous-flow anaerobic fermentors simulating the bovine rumen and the colon environment to model in the laboratory the population dynamics of the relationship between predator (phage) and prey (including nonpathogenic derivatives of E. coli 0157:147), facilitating selection of the most promising phages and diets for treatment and exploration of the parameters of phage-host interaction as they are affected by conditions and by the presence of other rumen and colon bacteria. Phage will be prepared and supplied to Dr. Todd Callaway of the USDA, who will extend these studies to pathogenic strains and to in vivo studies in cattle. Such experiments are very well suited to work by students in our undergraduate setting, leading to a firm foundation in understanding microbial ecology and physiology, general microbial and anaerobic techniques, proteomics, and experimental design, along with the satisfaction of contributing significant new research results.

Grant: 1R21GM063117-01
Program Director: CHIN, JEAN
Principal Investigator: MENON, ANANT K
Title: Phospholipid flip-flop in biogenic membranes
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 2001/04/01-2003/03/31

DESCRIPTION (applicant's abstract): In this R21 (exploratory/developmental) grant application, we wish to address a central problem concerning the assembly of the phospholipid bilayer of biological membranes, i.e., what is the mechanism by which phospholipids are translocated across biogenic (self-synthesizing) membranes? This is an underappreciated, understudied area, and we believe that despite the intrinsic experimental difficulties in measuring phospholipid flip-flop, we are in a unique position to approach this longstanding problem. Newly synthesized phospholipids are located in the cytoplasmic leaflet of biogenic membranes such as the endoplasmic reticulum (ER) of eukaryotic cells and the cytoplasmic membrane (bCM) of bacteria. While these lipids can diffuse laterally in the membrane leaflet in which they are situated, transverse diffusion or flip-flop is thermodynamically restricted and does not occur spontaneously. However, such movement is essential to propagate a membrane bilayer, and a number of reports indicate that phospholipids translocate rapidly across the ER and bCM. These reports also indicate that translocation is bidirectional and occurs by a facilitated diffusion process requiring no metabolic energy input. This observation rules out the participation of the ABC family of transporters which are involved in metabolic energy-dependent, vectorial transport of solutes and some lipids. Thus the molecular mechanism by which phospholipids are translocated across the ER and bCM is unknown. We hypothesize that there exists a novel class of lipid translocators that facilitate diffusion of phospholipids in a metabolic energy-independent fashion across the ER and bCM bilayers. We term these translocators biogenic membrane flippases, to distinguish them from metabolic energy-requiring lipid translocators (like the ABC transporters) that are driven by ATP hydrolysis or protonmotive force. Our aim is to identify a biogenic membrane flippase by biochemical and genetic means, thus providing a direct test of our hypothesis. To do this we will explore biochemical reconstitution/protein purification approaches using *Bacillus* membranes as a starting point, as well as forward and reverse genetic strategies utilizing *Escherichia coli*. The last of these involves a comparative genomics approach, making novel use of available sequenced genome databases. Our long term goal is to obtain a molecular definition of the mechanism of phospholipid flip-flop. We expect that our analyses will not only impact current understanding of membrane biogenesis, but also contribute to an understanding of glycolipid flip-flop events that are essential in the assembly of cell surface components.

Grant: 2R37GM034496-17

Program Director: CHIN, JEAN

Principal Investigator: ROCK, CHARLES O

PHD

BIOCHEMISTRY:BIOCHEM

RY-UNSPEC

Title: REGULATION OF LIPID METABOLISM IN BACTERIA

Institution: ST. JUDE CHILDREN'S RESEARCH HOSPITAL MEMPHIS, TN

Project Period: 1984/12/01-2005/11/30

DESCRIPTION (Adapted from applicant's abstract): The long-term goal of this project is to understand the mechanisms that regulate bacterial membrane lipid biosynthesis and explore the structure, function and diversity of the enzymes involved in this pathway. The study of *Escherichia coli* has historically served as the paradigm for bacterial lipid metabolism. The evolution of lipid biosynthesis as a focal point for the development of novel therapeutics and the availability of a wealth of genomic sequences has stimulated the exploration of these pathways in important pathogens. The discovery of two novel enoyl-[acyl carrier protein] reductases in Gram-positive bacteria during the last grant period highlights the importance of this avenue of research. We have developed the tools for a multidisciplinary attack on this important problem that will incorporate the techniques of structural biology into all facets of the research. The research plan builds on the important discoveries made during the last grant period and is organized into three subject areas. The enoyl reductase step is a key regulator of fatty acid elongation and the target for widely used antibacterial agents. In the first aim, we will investigate the biochemical mechanism, structure and function of the enoyl reductase and expand this work to include the two newly discovered enoyl reductases of Gram-positive bacteria as well as the universally expressed and highly conserved beta-ketoacyl-[acyl carrier protein] reductase. Lipid metabolism is a vital facet of bacterial physiology and in the second aim we will define the regulatory mechanisms that integrate fatty acid biosynthesis into cell physiology and coordinate membrane lipid formation with macromolecular biosynthesis. Our investigation of fatty acid biosynthesis in Gram-positive bacteria will focus on elucidating the pathways for unsaturated fatty acid synthesis in an important pathogen. The condensing enzymes are key regulators of fatty acid composition, and in the third aim, we will define the molecular characteristics that determine their substrate specificity, use these enzymes as a model for defining the critical 3-dimensional features necessary for the docking of acyl carrier protein, and determine the mechanism of action of a broad-spectrum antibiotic, thiolactomycin. The results of these investigations will provide important new information on the structure, function, diversity and regulation of fatty acid biosynthesis that will contribute to the basic understanding of bacterial physiology and complement the development of novel antibacterial therapeutics.

Grant: 2R37GM038660-15
Program Director: TOMPKINS, LAURIE
Principal Investigator: LANDICK, ROBERT C
Title: Structure/Function of Transcription Complex RNA Hairpins
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 1987/07/01-2006/06/30

DESCRIPTION (provided by applicant): The long-range goal of this project is to define the interactions in the transcription complex that regulate pausing and termination by RNA polymerase. Nascent RNA hairpins are important regulatory signals in bacteria, where pausing and termination are major components of genetic regulatory mechanisms. Pausing and premature termination also affect expression of genes in mammalian cells and viruses, notably genes involved in the development of cancer and in growth of the AIDS virus, HIV- 1. In both bacteria and eukaryotes, specialized regulatory proteins modify the transcription complex to make it resistant to pausing and termination. Although significant progress has been made in understanding pausing, termination, and the regulatory proteins that control these events, two alternative models remain possible. In one view, called the allosteric model, pause signals, termination signals, and regulatory proteins primarily affect the conformation of RNA polymerase. In the other, these signals and proteins primarily affect translocation of a relatively rigid RNA polymerase on the RNA and DNA chains (the rigid-body model). Pausing and termination by *E. coli* RNA polymerase and their regulation by the NusA, NusG, and RfaH proteins, and pausing by human RNA polymerase II have been developed as model systems. A combination of biochemical, genetic, and biophysical approaches will be used to distinguish the allosteric and rigid-body models of transcriptional regulation, and to characterize the mechanisms of pausing, termination, and regulatory proteins that control them. Specific aims will be to (i) characterize interactions of RNA polymerase's flap-tip helix with RNA, NusA, and sigma-70, and test how these interactions affect catalysis in the active site; (ii) determine the location of the RNA 3' about end in paused and nonpaused transcription elongation complexes; (iii) determine the kinetic mechanisms of elongation, pausing, and termination; (iv) map interactions between RNA polymerase and pause and terminator hairpins; and (v) determine the sites at which RfaH and NusG interact with RNA polymerase and the mechanisms by which they regulate transcript elongation.

Grant: 2R37GM038784-14
Program Director: PREUSCH, PETER C.
Principal Investigator: O'HALLORAN, THOMAS V
Title: METALLOREGULATION BY MERR AND FUR PROTEIN FAMILIES
Institution: NORTHWESTERN UNIVERSITY EVANSTON, IL
Project Period: 1987/07/01-2006/02/28

DESCRIPTION (applicant's description): Metal ion sensory mechanisms are critical for cellular responses to essential and toxic metals alike. Emerging from studies of microbial metalloregulatory systems are general models that serve as starting points for understanding the cell biology of metals in humans. The MerR and Fur families of metalloregulatory proteins control the expression of an array of genes that protect the eubacterial cell from physical and chemical stresses including antibiotic treatments. In a variety of virulent microbes, Fur or a closely related iron-sensor protein controls toxin expression. A general but controversial mechanism for iron-responsive derepression has been proposed but is as of yet unresolved. Mechanistic studies of these mercury and iron sensor proteins are now beginning to provide insights into zinc and copper-responsive metalloregulation. The *E. coli* ZntR protein, a recently discovered member of the MerR family, is a zinc-specific metalloregulatory protein that controls expression of zinc export machinery. Its counterpart, the Zur protein, is a member of the Fur family that exerts zinc-responsive control over the expression of zinc uptake machinery. Together these genes govern zinc uptake and export, ensuring that cells experience neither zinc starvation nor toxicity. In both cases the mechanisms of transcriptional control or the molecular basis of metal recognition are not yet established. This proposal focuses on energetic and structural aspects of metal recognition and metal-induced conformation changes in the allosteric switching mechanism. MerR controls transcription in an unprecedented manner: metal-protein interactions induce distortions in DNA structure that make the DNA a better template for the transcription machinery. By comparing the positive control mechanism for other family members such as ZntR, a comprehensive test of this DNA distortion mechanism is possible. Positive control mechanisms are poorly understood and yet are of fundamental importance in understanding the molecular basis of genetic regulation. The molecular basis of heavy metal recognition in the ZntR, Zur, and Fur systems will be probed at the biopolymer and coordination chemistry levels. The structure, function, and energetic insights of these new stress-responsive transcription factors will provide a deeper understanding of molecular mechanisms and transition metal cell biology.

Grant: 2R37GM039422-14

Program Director: RHOADES, MARCUS M.

Principal Investigator: BELFORT, MARLENE PHD

GENETICS:BIOCHEMICAL/M

LECULAR

Title: INTRON DYNAMICS IN BACTERIA

Institution: WADSWORTH CENTER ALBANY, NY

Project Period: 1988/02/01-2006/01/31

DESCRIPTION: Group I and group II introns are dynamic genetic elements that splice by different self-catalyzed RNA-based mechanisms. Many are also capable of insertion into DNA through distinctive mobility pathways. For each class of intron, mobility characteristically takes the form of homing, whereby the intron transfers to an intronless DNA allele via a break created by an intron-encoded endonuclease. For group I intron homing, recombination events are strictly DNA-based, whereas group II intron homing, also termed retrohoming, involves RNA at levels of both the template and cleavage enzyme for mobility. The overall goal of this work is to study in bacteria the DNA-based and RNA-based intron rearrangements of these phylogenetically diverse elements. During the past funding period the PI made progress in understanding the role of DNA exonucleases in group I intron mobility, and in demonstrating illegitimate double-strand-break repair in intron acquisition, suggesting that such a mechanism might account for intron invasion of ectopic sites. In independent studies, the PI defined the domain structure and function of StpA, an RNA chaperone that promotes splicing of group I introns. The PI also determined the mode of action of DsrA, an E. coli regulatory RNA. Finally, in major breakthroughs for the field, the PI established a group II intron as the first functional retrotransposon in prokaryotes demonstrating both RNA-based retrohoming and retrotransposition to ectopic sites. In addition to the innate mechanistic importance of these results, the invasiveness of group II introns and their similarities to human retrotransposons and spliceosomal introns have great evolutionary and health significance. For the next funding period the PI proposes to demonstrate transposition of group I introns. Additionally, the PI will extend her analysis of the RNA chaperone function of StpA and further define its structure and global regulatory activity. The PI will also build on her mechanistic studies and define accessory molecules required for group II intron retrohoming and retrotransposition. Finally, the PI will ask evolutionary questions relating to group II intron dispersal, the relationship of group II introns to spliceosomal introns of eukaryotes, and the possible role of group II introns in affecting horizontal gene transfer. Thus, by combining the approaches of genetics, biochemistry, and structural analysis, the PI proposes to advance our understanding of intron-related nucleic acid dynamics and intron evolution.

Grant: 1P01HD040539-01
Program Director: REICHELDERFER, PATRICIA
Principal Investigator: LANDAY, ALAN L
Title: FEMALE GENITAL TRACT IN HIV PATHOGENESIS
Institution: RUSH UNIVERSITY MEDICAL CENTER CHICAGO, IL
Project Period: 2001/04/23-2006/03/31

The program project " Genital Tract in HIV pathogenesis" will investigate immunologic, and host factors in the female genital microenvironment that influence HIV infectivity and susceptibility. This program project integrates three research projects and three Core facilities. Subproject 0001, "Bacterial Vaginosis (BV) and HIV in the Female Genital Tract" proposes that BV in the genital tract increases HIV susceptibility and will assess the effects of organisms associated with BV on HIV expression. Subproject 0002, "Female Genital Tract Immunity and HIV vaccine responses" focuses on HIV specific vaccination, and the role of pre-existing genital tract HIV immunity in the response to immunization. Subproject 0002, "HIV-1 Susceptibility Factors in Cervical Secretions" will focus on the microbiology/immunology of the cervical microenvironment and how secreted and microbial factors inhibit or enhance susceptibility to HIV infection in the female genital tract. The projects will utilize the well established HIV cohorts (REACH, WIHS, WITS) spanning ages from adolescence to adulthood and the spectrum of HIV infection from asymptomatic to AIDS as well as two high risk seronegative cohorts. The research projects are supported by three Cores: Statistics and Data Management Core coordinating research, scientific meetings and data management support; the Clinical Core managing subject recruitment, existing cohort utilization and clinical data collection; and the Laboratory Core establishing a specimen repository and providing virologic and immunologic assays. By characterizing factors affecting HIV infectivity and susceptibility to infection, the studies will increase our understanding of sexual and perinatal HIV transmission and contribute to future therapy and vaccine strategies.

Grant: 1R01HD038856-01A1
Program Director: WILLINGER, MARIAN
Principal Investigator: NELSON, DEBORAH B PHD
Title: Bacterial Vaginosis and Spontaneous Abortion
Institution: UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA
Project Period: 2001/06/01-2005/03/31

DESCRIPTION: Bacterial vaginosis (BV) is extremely prevalent among low-income, urban pregnant women. The current standard of medical care does not involve screening pregnant women for BV unless clinical symptoms are reported; however, the majority of pregnant women with BV are asymptomatic. A number of studies have found associations between BV and late pregnancy outcomes; such as, preterm labor, premature rupture of membrane, chorioamnionitis, and low birth weight. The impact of BV on the risk for spontaneous abortion (SAB) is unclear. In the proposed prospective cohort study, all women will be screened for BV early in pregnancy regardless of symptoms. The specific aims of this study are to: 1) characterize the prevalence and predictors of BV in women early in pregnancy and 2) evaluate whether BV during pregnancy is an important, independent predictor of SAB. Women attending their first clinical prenatal care visit at the Hospital of the University of Pennsylvania Obstetric Clinic with a pregnancy of 12 weeks gestation or less as determined by last menstrual period will be recruited. We will screen all women for bacterial vaginosis and follow-up through 22 weeks gestation to identify women experiencing a spontaneous abortion. We will enroll 2200 women over a three year period and compare SAB rates for the estimated 400 women found to test positive for BV (20 percent of patients) and the 1600 women found to test negative for BV. Baseline data collection will be standardized and include a structured in-person interview, a vaginal smear used to detect BV, and urine analysis to determine alcohol, cocaine and cotinine. Follow-up telephone interviews will be conducted at 22 weeks gestation to determine the status of pregnancy (SAB vs. non-SAB) and BV diagnosis and treatment. Pregnancy outcome status will also be ascertained through ongoing review of medical records, pathology logs and birth certificates. Initial analyses will be exploratory and descriptive, characterizing the prevalence and predictors of BV and the risk factors for SAB. The primary analysis will be logistic regression, with relative risks and 95 percent confidence intervals, to explore whether BV is an independent predictor of SAB. This study will provide a unique opportunity to evaluate the prevalence of BV among pregnant women and to determine the relationship between BV and incident SAB.

Grant: 1R01HD039455-01A1
Program Director: PARROTT, ESTELLA C
Principal Investigator: FUNKHOUSER, ELLEN M DRPH
Title: DOUCHING AND REPRODUCTIVE TRACT INFECTIONS
Institution: UNIVERSITY OF ALABAMA AT BIRMINGHAM, AL
BIRMINGHAM
Project Period: 2001/06/01-2004/05/31

Douching is a common practice among American women, especially in the South, among Black women, and among women who are less educated. Douching has been associated with many adverse health events including pelvic inflammatory disease and ectopic pregnancy, and to a much less well established degree, sexually transmitted diseases (STDs). The proposed project is a cross-sectional study of reproductive tract infections and douching practices in Jefferson County, AL. Women attending the County STD clinic and 2 County Family Planning Clinics will be interviewed prior to examination regarding douching practices and history of sexual activities, pregnancies, contraceptive practices, and STDs. Presence of infections and pH of vaginal secretions will be ascertained from appropriate tests. Cases will be women presenting with syphilis, gonorrhea, trichomonas, chlamydia, or bacterial vaginosis. Over a 29 month period 4,370 women, 1,400 from the STD clinic and 2,970 from the Family Planning Clinics, will be interviewed. This should provide about 935 STD cases, 577 cases of bacterial vaginosis without an STD, and 2,858 women with no infections. Douching practices among women with and without a reproductive tract infections will be compared. Logistic regression analysis will be used to assess the following: 1) whether douching is associated with increased risks of STDs or bacterial vaginosis; 2) whether douching is associated with vaginal pH; 3) whether there is a dose-response relationship regarding frequency of douching; and 4) whether the risk differs according to preparation used. We believe the similarities in socioeconomic status of women attending the clinics will be substantial making douching practices potentially one of the most distinguishing characteristics of women with and without an infection. Furthermore, the findings will be readily generalizable to a population that historically and currently has some of the highest STD rates in the nation.

Grant: 1R01HD039633-01A1
Program Director: NEWCOMER, SUSAN
Principal Investigator: ROGERS, SUSAN M PHD OTHER AREAS
Title: Transmissibility of GC and Ct Diagnosed using NAAT
Institution: RESEARCH TRIANGLE INSTITUTE RES TRIANGLE PK, NC
Project Period: 2001/09/27-2005/02/28

DESCRIPTION: (provided by applicant) Infections with *Neisseria gonorrhoeae* (GC) and *Chlamydia trachomatis* (Ct) are known to facilitate HIV transmission. It is estimated, for example, that the presence of untreated chlamydial infection increases the likelihood per contact of HIV transmission by a relative risk of 3 about 6. These STDs have other important health consequences, including pelvic inflammatory disease, perinatal complications, and subsequent risks of infertility and ectopic pregnancy in women. The development of nucleic acid amplification tests (NAAT) that can be used with urine specimens has permitted a new paradigm for epidemiological research on these STDs. Urine specimens for STD detection can easily be obtained in population surveys and public health programs to enable generalizations about the prevalence of symptomatic and asymptomatic infections in the population at large and among hard to reach at-risk populations. Results of these studies are disturbing. In one major U.S. city, for example, it is estimated that 8.3 percent of adults ages 18-35 has an untreated GC or Ct infection. Most of these infections were diagnosed among adults who report no recent symptoms and who do not present the classic STD behavioral risk profile. These results could indicate a large and hidden epidemic of asymptomatic infections that are unlikely to be detected and treated without vigorous public health interventions. However, it is also possible that NAAT testing is identifying clinically inconsequential infections because of the assay's ability to detect extremely low levels of viable organisms (i.e., below the infectious inoculum) or amplifiable DNA (or RNA) from residual pathogens (i.e., non-viable organisms) of past infections that are well on their way to being cleared. We propose to explore this issue by testing a sample of 8,000 adults, ages 18-35, attending the Johns Hopkins Adult Emergency Department. Subjects testing positive for GC or Ct will be re-evaluated using traditional diagnostic tests for these infections and be treated. Recent sexual partners of infected subjects and a random subsample of partners from uninfected subjects will also be contacted and tested. The proposed research will allow us to: (1) determine whether the probabilities of infection transmission are equivalent for GC and Ct infections detectable only by NAAT versus infections detectable by traditional testing procedures; (2) determine whether asymptomatic infections have an equivalent probability of transmission as symptomatic infections; (3) determine whether infections that can only be detected by NAAT testing have the same clinical consequences as infections that are detectable by traditional assays; and (4) examine the correlates of infections detected by NAAT versus traditional diagnostic tests.

Grant: 1R01HD040772-01
Program Director: KAUFMAN, STEVEN
Principal Investigator: LIAO, ZHAOHAO
Title: CYCLODEXTRIN AS NOVEL SPERMICIDE AND MICROBICIDE
Institution: JOHNS HOPKINS UNIVERSITY BALTIMORE, MD
Project Period: 2001/04/27-2004/03/31

DESCRIPTION (provided by applicant): Locally applied biomedical barriers and microbicides have proven ineffectual in preventing sexual transmission of HIV. We have recently found that HIV requires intact lipid rafts, highly specialized subregions in cell membranes, for entry into cells and for budding of fully infectious particles. Beta-cyclodextrin (beta-CD), a cyclic heptasaccharide that removes cholesterol from cell membranes and disperses lipid rafts, has been shown to block HIV infection and drastically reduce the infectivity of budding HIV particles. Cholesterol is also required by other pathogens some of which are associated with STDs. Beta-cyclodextrin is non-toxic and currently in human use as a carrier for polar drugs. Thus properly formulated, this molecule may be an effective microbicide with activity against HIV and other pathogens. Cholesterol has been shown to play a role in regulating sperm cell acrosomal reaction and depletion of sperm cell cholesterol by beta-CD induces capacitation and premature acrosomal reaction. The latter has been associated with low rates of fertilization. Thus beta-CD, by depleting sperm cell cholesterol, may prevent fertilization in vivo by inducing premature acrosomal reactions and reducing fusion efficiency. The central hypothesis of this proposal is that because it rapidly and efficiently depletes cholesterol from lipid membranes beta-CD has great potential as a combination microbicide-spermicide with low host cell toxicity. The goal of this project is to use in vitro and in vivo animal models to test the potential of beta-CD as a microbicide, particularly against HIV, and spermicide and to determine the mechanisms by which beta-CD inactivates HIV. The specific aims are: 1. To characterize and optimize the inhibitory effect of Beta-cyclodextrin on HIV-1 infection. 2. To determine the mechanisms by which Beta-cyclodextrin inactivates cell-free HIV-1 particles. 3. To determine the mechanisms by which Beta-cyclodextrin inactivates cell-associated HIV-1. 4. To determine the effects of Beta-cyclodextrin on sperm function in a rabbit contraception model. 5. To determine the effect of Beta-cyclodextrin in vitro on bacterial pathogens and vaginal flora. 6. To determine the anti-HIV microbicide potential of Beta-cyclodextrin in a Hu-PBL-SCID mouse vaginal challenge model. 7. To determine the microbicide potential of Beta-cyclodextrin against HSV, papillomavirus, and chlamydia trachomatis in animal models. This project takes advantage of the shared importance of cholesterol in the biology of sperm and HIV and other pathogens to develop a novel microbicide/contraceptive approach.

Grant: 1R01HD041682-01
Program Director: SPONG, CATHERINE
Principal Investigator: HITTI, JANE E AB
Title: Mechanisms for Racial Disparity in Preterm Birth
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2001/09/24-2006/07/31

DESCRIPTION (provided by applicant): We propose a five year population-based prospective cohort study to evaluate the relative contributions to pre term birth of 10 genital tract infection, maternal stress and a genetic predisposition to an enhanced immune response among African American and white women resident in King County, Washington. Potential subjects will be identified through birth certificate data, with appropriate measures to protect confidentiality. We will enroll 100 African American and 100 white women with a prior early preterm birth at 20-34 weeks gestation and a comparison group of 100 African American and 100 white women with prior term birth at >36 weeks. The initial assessment will be performed at least 6 months after the index delivery and will include evaluation of vaginal flora and endometritis, maternal stress by qualitative and quantitative measures, periodontitis, and genetic variability in cytokine production. We will offer participants treatment or referral for any modifiable risk factors for preterm birth that are identified in the initial evaluation. We will then follow subjects prospectively and anticipate that 30-40% of the cohort will have a subsequent pregnancy during follow-up. Women with a subsequent pregnancy will be offered evaluation of vaginal flora, cervical length, and maternal stress with treatment or referral offered for modifiable risk factors. Outcomes for second pregnancies will be ascertained. This study design will allow us to examine the following specific aims: 1. Study the role of increased antigenic stimulation from lower genital tract infection as a determinant of endometritis, chorioamnionitis and preterm birth among African American and white women. 2. Examine the correlation of maternal stress with inflammatory arousal, stratified by race and prior pregnancy history. 3. Assess maternal and fetal genetic contributions to the pro-inflammatory response and correlate these with preterm birth and neonatal outcome. In combination, these inter-related aims will address the most plausible mechanisms by which African American women continue to be at least twice as likely as white women to deliver prematurely. We also plan to explore the synergy between genetic predisposition, maternal stress, inflammatory arousal, lower genital tract infection, and preterm birth. We hypothesize that women with more than one predisposing factor are at a markedly increased risk for preterm birth, and that African American women are more likely than white women to have multiple predisposing factors. We hope that these studies may eventually lead to the development of more effective strategies to prevent preterm birth and to reduce the disparity in preterm birth, low birthweight and infant mortality between African American and white women.

Grant: 1R01HD041687-01
Program Director: SPONG, CATHERINE
Principal Investigator: NOWICKI, BOGDAN J
Title: CD556, Infection, and Race in Preterm Delivery
Institution: UNIVERSITY OF TEXAS MEDICAL BR GALVESTON, TX
GALVESTON
Project Period: 2001/09/24-2006/07/31

DESCRIPTION (provided by applicant): The rate of preterm delivery among African American (AA) women is 1.5 to 2.4 times higher than Caucasian (CS). The 2010 national public health goal is eliminating ethnic disparity in preterm delivery (PTD). Although the mechanism of PTD is unclear, infections are among the main ethnologic factors implicated. We propose here a unifying hypothesis by which infection and host factors may increase the risk of PTD among African Americans (AA). The fetus is a semiallogenic antigen that requires efficient protection from the maternal humoral immune system. Decay accelerating factor (DAF) is a protective host factor DAF is expressed in the feto-maternal interphase and its main function is protection from cytotoxic effect of autologous complement (c)attack. DAF expression is controlled by progesterone (P) and P was implicated in pregnancy losses. Infection upregulates nitric oxide (NO) and in turn NO downregulates DAF expression. Decreased DAF expression alters the protective quality of the feto-maternal interphase and may trigger complement mediated increases in proinflammatory tumor necrosis factor (TNF), and prostaglandin production resulting in PTL/PTD. We propose that both infection and biologic/genetic factors may contribute to the disturbances in the endocrine-NO-immune axis. Various factors in concert may act to alter the C/DAF ratio in the feto-maternal interface thereby leading to the activation of proinflammatory/prostaglandin pathway. The C cascade and TNF response occurs upon infection or vaginal colonization. Virulent E. coli is capable to display synergistic signaling via E.coli adhesin-host receptor and elicit aggressive cytokine responses. Although these factors may effect all women, AA are at higher risk due to an increased inherent capacity of rejection or hyperresponsiveness of alloantigens. The inherent differences may result in the observed PTD disparities between AA and CS. We propose to the following: 1. Characterize expression of DAF among AA and CS women undergoing elective pregnancy tennination, term and pretenn labor. 2. Characterize allelic polymorphism in the DAF Tcb, Cr(a-), and TNFA alleles and analyze possible association with infection, PTD and race. 3. Characterize genotypes of vaginal colonization isolates in patients with term and preterm delivery. The long-term goal of our project is to characterize mechanism of infection associated PTL/PTD and the role of the host and pathogen factors interacting at the urogenital interphase in birth outcome.

Grant: 1U01HD040574-01
Program Director: WRIGHT, LINDA
Principal Investigator: PANIGRAHI, PINAKI MD
Title: Prevention of Infection in Indian Neonates
Institution: UNIVERSITY OF MARYLAND BALT PROF SCHOOL BALTIMORE, MD
Project Period: 2001/09/03-2006/04/30

DESCRIPTION (Provided by applicant): India, with one of the world's largest populations, continues to struggle with extremely high infant and neonatal mortality rates. Sepsis now accounts for 50% of deaths among community-born (and 20% of mortality among hospital-born) infants. Closely linked with this is a burgeoning problem with antimicrobial resistance, which is increasingly restricting the therapeutic options for medical care providers. To deal with these critical issues, the investigators propose to establish a Research Unit for the study of MCH in India, based on strong, existing collaborations between investigators in the Department of Pediatrics and Epidemiology and Preventive Medicine at the UMSM, Baltimore, and the AIIMS, New Delhi, and hospitals and the Ministry of Health in the state of Orissa, India. The applicant will initially develop an infrastructure to monitor occurrence of neonatal sepsis in community- and hospital-born infants. This will include: 1) identification of all hospitalized children, and children brought to hospital, with the diagnosis of sepsis; 2) obtaining blood cultures from these children; 3) screening of all bacterial strains isolated from blood cultures for antimicrobial resistance; 4) collecting basic demographic, risk factor, and treatment data on each case; and 5) development of a computer-based system and network for data management. In villages of Orissa State, the applicants will set up a village-level surveillance system to identify women during their pregnancy, monitor pregnancy outcomes, and establish a mechanism for referral of all potentially septic infants to participating clinics or hospitals for evaluation, including collection of blood cultures. Subsequent studies will identify potential sources of bacterial isolates causing sepsis. To this end, the applicants will screen skin, nares, and stool cultures from infants (and skin, nares, and vaginal cultures from their mothers), and seek to match blood isolates with these colonizing isolates, using molecular epidemiologic techniques. In the latter years of the grant period, and with these data collection systems in place, the applicants will initiate a series of interventions, including implementation of a hospital- and community-based system of "preferred" antimicrobials, use of probiotics to reduce the risk of neonatal sepsis, and implement alcohol-based hand-washing products in hospital and community-based healthcare settings to minimize pathogen transmission.

Grant: 2P01HL043026-11A1
Program Director: KELLEY, CHRISTINE
Principal Investigator: SCHMID-SCHOENBEIN, GEERT W
Title: Biomechanics of the Heart, Vessels, and Blood Cells
Institution: UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA
Project Period: 1990/07/01-2006/05/31

Abstract Text Not Available

Grant: 1P01HL067672-01
Program Director: BANKS-SCHLEGEL, SUSAN P.
Principal Investigator: MARTINEZ, FERNANDO D MD
Title: SCOR IN CELLULAR AND MOLECULAR MECHANISMS OF ASTHMA
Institution: UNIVERSITY OF ARIZONA TUCSON, AZ
Project Period: 2001/09/30-2006/08/31

(Applicant's Abstract) Most cases of asthma begin during the first years of life. This suggests that a significant proportion of the risk for the development of asthma can be attributed to complex gene by environment interactions occurring during these early years. Understanding the developmental alterations of the immune system in early life that are associated with the subsequent development of asthma, is essential to designing a strategy for primary and secondary prevention of the disease. In this SCOR proposal we will integrate molecular, immunologic, genetic, genomic, and epidemiologic approaches to study the immune, genetic, and environmental interactions that occur at the beginning of asthma. In Project 1 we explore the gene by environment interactions that may be involved in the apparent protective effect of an increased microbial burden in early life on the development of early allergic sensitization and asthma. A population sample of children living in rural areas of Europe and in whom exposure to indoor endotoxin has been assessed will be studied. Known or newly discovered polymorphisms in genes coding for the main components of the endotoxin response system will be assessed and related to endotoxin exposure and asthma-related outcomes. In Project 2, the genetic and immune factors that explain the strong and independent relation between atopic dermatitis (eczema) in early life and the subsequent development of asthma will be explored. It is well known that most children who will go on to develop asthma show Th2-deviated responses to local aeroallergens very early in life, but not all children who do show such responses develop the disease. Project 3 will explore the complex molecular mechanisms that determine if a Th2-deviated response will result in the synthesis of IgG4, IgE or both in humans. Finally, we have recently described five new polymorphisms in the promoter region of the CD14 gene. We have shown that these polymorphisms are associated with total serum IgE levels in school children. Project 4 will explore the biology of novel proximal and distant regulatory elements of the CD14 gene and will thus provide new insights on the mechanisms by which the innate immune response may influence the susceptibility to early allergies and asthma. Our SCOR offers a unique opportunity to study in a comprehensive manner the way in which environmental factors and genetic background influence the maturation of the immune system during the initial phases of the asthma process.

Grant: 2R01HL046809-09A1
Program Director: PETERSON, CHARLES M
Principal Investigator: GANZ, TOMAS
Title: Human Defensins in Phagocytes and Epithelia
Institution: UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA
Project Period: 1993/01/01-2005/06/30

DESCRIPTION (provided by applicant): This proposal is a continuation of a highly successful program of characterization of a family of human host defense peptides, defensins. Accumulated evidence from studies in humans, as well as in animals and plants, strongly supports the important role of defensins in innate immunity. Most defensins at physiologic concentrations are antimicrobial and some defensins also have chemokine-like activities. In previous studies we characterized the abundant defensins from polymorphonuclear leukocytes but more recently we focused on other defensin peptides expressed in resting and inflamed epithelia. We will now examine the biology of the inflammation and infection-inducible epithelial human beta-defensin-2, and characterize the biologically-related human beta-defensin-3. Continuing with our studies of human neutrophil defensins, we will identify and characterize the prodefensin convertase, a key enzyme for the activation of neutrophil defensins and an attractive target for experimental ablation of defensin activity. We will: 1) Characterize the synthesis, cellular and subcellular distribution, and biological function of the inducible epithelial defensin HBD2; 2) Analyze the regulation of HBD2 response in organotypic epidermal culture and in a transgenic reporter mouse; 3) Biosynthesize the newly discovered beta-defensin HBD3 and characterize its bioactivities, its tissue distribution and its inducibility by infectious and inflammatory stimuli; 4) Identify and characterize the neutrophil defensin convertase, the enzyme responsible for defensin activation. In the aggregate, these studies will expand our understanding of this ubiquitous and multifunctional family of human host defense peptides, this time also in their role as effectors of innate immunity in epithelia.

Grant: 2R01HL052589-05A2
Program Director: MASSICOT-FISHER, JUDITH
Principal Investigator: MCGOWAN, FRANCIS X MD
Title: Myocardial Endotoxin Signaling in Surgery
Institution: CHILDREN'S HOSPITAL (BOSTON) BOSTON, MA
Project Period: 1996/03/01-2005/02/28

DESCRIPTION (Verbatim from the applicant's abstract) Release of bacterial endotoxin (lipopolysaccharide, LPS) into the circulation occurs in sepsis, as well as after major trauma and surgery. Sepsis alone affects more than 500,000 patients per year in the United States, nearly half of which die. LPS triggers a systemic inflammatory response and multi-organ dysfunction due to cellular activation of both immune and non-myeloid cells. Endotoxemia-induced myocardial dysfunction is an important determinant of morbidity and mortality in sepsis; it and related mechanisms have also been associated with the pathophysiology of heart failure and cardiopulmonary bypass. New information detailed in the present proposal and from other investigators indicates that both receptor mediated and direct intracellular trafficking of LPS are likely to be responsible for stimulating the intracellular signaling cascades, perturbation of cellular functions, and gene transcription events that are responsible for LPS-induced cellular injury. These effects occur quite rapidly (30-60 min) and include decreased myocardial contractility, abnormal calcium regulation, oxygen wastage, abnormal mitochondrial transport, and free radical production. These abnormalities coincide with initiation of multiple signaling pathways and the transport of LPS to intracellular sites including mitochondria, Golgi, and the contractile apparatus. This project using myocytes in culture and isolated perfused hearts, will test three hypotheses: 1) that LPS activates Toll-like receptors and related signal transduction proteins; 2) that membrane association and intracellular transport of LPS are responsible for signaling and functional effects; and 3) that LPS or LPS-activated signal transduction pathways cause mitochondrial dysfunction. Given the potential diversity of the mechanism of LPS signal initiation and the pleiotropic nature of subsequent cellular responses, defining the earliest mechanisms of LPS signaling is essential to the design of specific and effective treatment strategies. Furthermore, the results from these studies are likely to apply not only endotoxin but also to host responses to other foreign pathogen products, and, in a more general sense, to understanding the role of stimulation of innate and adaptive immunity in various forms of myocardial injury.

Grant: 2R01HL055936-06
Program Director: PEAVY, HANNAH H
Principal Investigator: RUSSELL, DAVID G PHD PARASITOLOGY, OTH
Title: THE ROLE OF THE GRANULOMA IN M. TUBERCULOSIS INFECTIONS
Institution: CORNELL UNIVERSITY ITHACA ITHACA, NY
Project Period: 1995/09/30-2006/03/31

DESCRIPTION: M. tuberculosis demonstrates an extraordinary penetrance of the human population. Much of its success is linked to its ability to persist within the host, which is dependent on the formation of granulomas, or localized infections that support bacterial persistence without overt disease. The granuloma fulfills functions ambivalent to the host because although it limits spread of the infection it also provides a haven for the bacterium from the more extreme rigors of the host immune response. Dr. Russell proposes to extend his existing studies into the life cycle stages of M. tuberculosis by studying how both the host and the bacterium initiate and maintain the granuloma in both the murine and human infections. The specific aims of the proposal are: 1. Characterization of the bacterial factors that induce and modulate granuloma development. M. tuberculosis synthesizes and releases 7 major lipids; these lipids induce granuloma-like structures in mice and stimulate a pro-inflammatory response in macrophages in culture. These lipids will be identified structurally and their biological activities delineated. 2. Elucidation of the roles of host cytokines, chemokines and their receptors in the biology of the granuloma. The ability of the cell wall lipids to induce granuloma in non-immune and immune mice will be determined and compared with bacterial granulomas. Bacterial lipid granulomas will be dissected in an in vivo model that mixes labeled macrophages from relevant knock-out mice with the lipid-bearing particles and the data compared to immunohistological analysis of murine tuberculosis granulomas. The PI will also develop an in vitro cell migration model to determine the cytokines and chemokines responsible for recruitment of macrophages to the infection foci. 3. Examination of human alveolar macrophages from BAL cells from tuberculosis patients. These studies will encompass functional characterization of BAL macrophages for phagocytosis, vacuolar acidification and cellular responsiveness. The PI will also examine the cytokine/chemokine profiles of these cells as well as whether or not they contain mycobacterial cell wall constituents. The BAL cells will also be examined in cell migration assays based on the result from the murine granuloma model described in aim # 2.

Grant: 2R01HL055967-06
Program Director: PEAUV, HANNAH H
Principal Investigator: BOOM, W HENRY
Title: M. TUBERCULOSIS INFECTION IN THE LUNG
Institution: CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH
Project Period: 1995/09/30-2006/05/31

DESCRIPTION (Adapted from the Applicant's Abstract): The lung is the major portal of entry for *Mycobacterium tuberculosis*, the cause of human tuberculosis. The lung is the site where immune responses to this bacterium are initiated, and where growth of the organism is controlled without complete eradication. Acquired immunity of T cells and macrophages controls infection in the majority of healthy individuals. The lung is not only the first site of infection, but also uniquely susceptible to *M. tuberculosis*. Mechanisms responsible for susceptibility of the lung to *M. tuberculosis* and the inability of acquired immunity to eradicate the bacteria are poorly understood. The murine model of aerogenic mycobacterial infection provides an excellent means to analyze the initiation and effector phases of acquired immunity to mycobacterial infection in the lung. This competitive renewal application for HL-55967 builds on the collaborative infrastructure developed during the last 4 years between Case Western Reserve University (CWRU) and Colorado State University (CSU) aimed at addressing immune mechanisms responsible for control of mycobacterial infection in the lung. The PI hypothesizes that mechanism(s) for the pulmonary susceptibility to *M. Tuberculosis* differ(s) according to stage and site of infection. Initially, *M. Tuberculosis* resists innate immune mechanisms in lung and uses alveolar and interstitial spaces as privileged sites. As acquired immunity develops, the ability of *M. Tuberculosis* to inhibit the function of antigen-processing cells becomes a dominant means to assure its survival within the lung. This hypothesis leads to the following specific aims: Aim1: To determine the mechanism(s) responsible for permissive mycobacterial growth in alveolar and interstitial lung macrophages and the role of chemokines and NK cells in control of mycobacterial growth during the innate phase of pulmonary *M. Tuberculosis* and *M. bovis* BCG infection. Aim2: To determine the ability of lung antigen presenting cells (alveolar macrophages, lung parenchymal macrophages and lung dendritic cells) to activate naive and memory T cells, and the mechanism(s) used by *M. Tuberculosis* to interfere with class II MHC antigen presenting cell function. Aim 3: To determine the ability of chemokines, CpG and cholera toxin to enhance innate and acquired immune defenses to *M. bovis*-BCG and *M. Tuberculosis* within lung.

Grant: 2R01HL056181-06
Program Director: LINK, REBECCA
Principal Investigator: BOCK, PAUL E PHD BIOLOGICAL
CHEMISTRY
Title: Molecular Mechanisms of Fibrinolysis
Institution: VANDERBILT UNIVERSITY NASHVILLE, TN
Project Period: 1996/07/01-2005/06/30

DESCRIPTION (provided by applicant): The long-term goal of the proposed studies is to determine the molecular mechanism by which streptokinase (SK) activates the human fibrinolytic system, which is the basis for the use of SK as a thrombolytic drug for the treatment of cardiovascular disease. The studies address significant gaps in the understanding of the unique mechanism of conformational activation of plasminogen (Pg) by SK and the coupled proteolytic activation pathway that converts Pg into the fibrinolytic proteinase, plasmin (Pm). In the hypotheses to be evaluated, conformational activation of Pg is triggered by rapid binding of SK and insertion of the sK aminoterminal into a binding pocket on Pg, in cooperation with stabilization of the activated conformation of the catalytic domain of the zymogen as a result of its preferentially higher affinity for SK. This initiates the proteolytic activation pathway by expression of a new binding site, first on SK-Pg and subsequently on SK-Pm complexes, that functions as an exosite to enable specific binding and cleavage of Pg as a substrate. Conformational and proteolytic activation of Pg by SK are modulated by intrinsic differences in the affinities of SK for ysJPg, and ysJPm, enhanced by involvement of lysine binding sites, and regulated in vivo by fibrinogen- and fibrin-promoted assembly of productive complexes. The hypotheses will be evaluated in quantitative equilibrium binding studies employing unique fluorescent derivatives of Pg, Pm, and SK, in combination with steady-state and rapid-reaction kinetics, and protein structural approaches. Specific aims are: (1) To define the sequence of molecular events in the mechanism of conformational activation of Pg induced by SK binding. (2) To delineate the mechanism of the SK-initiated proteolytic activation pathway of Pm formation. (3) To determine the functional roles of the SK alpha, beta, and gamma-domains and individual lysine residues of SK in the mechanisms of conformational and proteolytic activation of Pg. (4) To elucidate the mechanism of fibrinogen and fibrin regulation of SK-initiated fibrinolysis and its role in the fibrin-specificity of SK therapy. The proposed studies of fundamental thermodynamic, kinetic, and structural aspects of the mechanism are expected to change the current conceptualization of the mechanism of action of SK as a thrombolytic drug. New information derived from these studies may enable more fibrin-specific thrombolytic agents to be developed.

Grant: 1R01HL064039-01A1
Program Director: NOEL, PATRICIA
Principal Investigator: BOITANO, SCOTT A PHD
Title: MODELING AIRWAY RESPONSE TO BORDETELLA SP INFECTION
Institution: UNIVERSITY OF WYOMING LARAMIE, WY
Project Period: 2001/02/15-2004/01/31

DESCRIPTION (Applicant's Abstract): Although vaccination introduced in the late 1940s has been effective in reducing the incidence of Bordetella disease in humans, the disease is still endemic, and affects more than 60 million people annually. In the United States, the reported incidence of Bordetella disease is low, with 2,000 - 6,000 diagnosed cases each year. However, this number is an underestimate due to the difficulty of diagnosis, and Bordetella disease has been steadily increasing in immunocompromised and adult populations since the late 1970s. This research is directed at the understanding of Bordetella derived factors and their impact on the initiation and the progression of airway cell pathogenesis. The interaction between bacterial pathogens and their hosts can range from the establishment of a commensal relationship to a potentially lethal disease. During pathogenesis, bacteria continually monitor their environment and adjust virulence gene expression accordingly. It is thought pathogens from Bordetella sp. express specific gene products that allow for preferential binding of ciliated cells in the airway epithelium. Furthermore, it is suggested that this binding initiates host cell signaling pathways including transient changes in intracellular Ca^{2+} concentration ($[\text{Ca}^{2+}]_i$) and host cell cytoskeleton rearrangements that help establish a favorable local environment for the establishment and progression of Bordetella sp. infection. In this proposal, primary cultured rabbit tracheal epithelial cells and B. bronchiseptica strains genetically locked in specific virulence states or expressing selective virulence genes will be used to model cell pathogenesis in initiating Bordetella sp. infection. Video microscopy will be used to assay changes in physical attachment, digital imaging microscopy to assay changes in host cell signaling and immunocytochemistry to assay changes in the host cell cytoskeleton associated with specific gene products produced by Bordetella sp. during cell pathogenesis. A greater understanding of bacterial/host interactions and their resulting physiological significance should lead to better development of prevention therapies and treatment strategies against bacterial invasion.

Grant: 1R01HL064883-01A2
Program Director: ERSHOW, ABBY
Principal Investigator: ZHONG, GUANGMING PHD
Title: Exacerbation of Atherosclerosis
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR SAN SAN ANTONIO, TX
ANT
Project Period: 2001/07/01-2005/06/30

DESCRIPTION (provided by the applicant): Atherosclerosis is the principal contributor to the pathogenesis of myocardial and cerebral infarction, and no effective treatment is yet available for atherosclerosis. Identifying the etiological causes and understanding the pathogenic mechanisms of atherosclerosis may provide information for developing efficient strategies for preventing and/or controlling cardiovascular disease pathogenesis. Both epidemiological investigations and animal model studies have linked *C. pneumoniae* infection to atherosclerosis. The objectives of the present proposal are to further evaluate the role of *C. pneumoniae* infection in atherosclerosis in a mouse model system and to use this animal system to understand the mechanisms of *C. pneumoniae* atherogenesis by testing an infection / inflammatory response hypothesis. Specifically, the roles of both inflammatory responses and lipoprotein oxidation in *C. pneumoniae* exacerbation of atherosclerosis will be evaluated. These studies may provide the essential information on designing effective approaches for preventing and controlling *C. pneumoniae*-exacerbated atherosclerosis.

Grant: 1R01HL064884-01A1
Program Director: PEAVY, HANNAH H
Principal Investigator: KORNFELD, HARDY MD MEDICINE
Title: ANTIMYCOBACTERIAL MECHANISMS OF APOPTOSIS IN THE LUNG
Institution: BOSTON UNIVERSITY MEDICAL CAMPUS BOSTON, MA
Project Period: 2001/01/01-2005/12/30

DESCRIPTION (Applicant's Abstract): Innate antimicrobial mechanisms of resident alveolar macrophages (AM) and control bacterial replication early in pulmonary tuberculosis, prior to the expression of adaptive immunity. The goal of this project is to study how AM suppress growth of Mycobacterium tuberculosis (Mtb). Our preliminary data indicate that growth of intracellular mycobacteria is efficiently restricted when AM undergo apoptosis, whereas necrosis promotes unrestricted extracellular bacterial replication. Apoptosis induced by several different pathways has been linked to this antimicrobial effect by other investigators as well, but a mechanism of this effect has not been proposed. We previously discovered that infection with Mtb directly activates TNF α death signaling in AM which we postulate limits bacterial growth by i) sequestering bacilli in apoptotic bodies and ii) marking infected cells for engulfment by phagocytes that recognize apoptotic epitopes. Phagocytosis of free Mtb is associated with arrested phagosome maturation and unrestricted intracellular growth of bacilli. We hypothesize that mycobacteria packaged by apoptosis are attacked by intracellular antimicrobial effector systems more effectively than occurs when free bacilli are internalized. It has recently been discovered that human dendritic cells efficiently present antigen derived from apoptotic cells by a process called "antigen cross-priming." Experiments in this project examine cooperative antimicrobial when naive AM are presented with Mtb by apoptotic AM, a process that we call "pathogen cross-priming." This system mimics events that occur in the lung in vivo where initial infection of AM by Mtb promotes recruitment of naive AM and other mononuclear phagocytes to the site of infection. The cellular requirements, apoptosis-specific epitopes, and intracellular effector mechanisms triggered by these interactions will be investigated. Together, these studies will characterize a novel host defense mechanism in tuberculosis that may represent a fundamental process relevant to a variety of intracellular lung pathogens.

Grant: 1R01HL065509-01A1
Program Director: BANKS-SCHLEGEL, SUSAN P.
Principal Investigator: SACHDEV, GOVERDHAN P PHD
Title: Biochemical Role of Airway Mucins in Cystic Fibrosis
Institution: UNIVERSITY OF OKLAHOMA HLTH SCIENCES CTR OKLAHOMA CITY, OK
Project Period: 2001/08/01-2004/07/31

DESCRIPTION (provided by applicant): Cystic Fibrosis (CF) is the most lethal genetic disease in Caucasians and is characterized by production of excessive amounts of viscous mucus secretions in the airways of the patient. This causes airway obstruction as well as chronic bacterial infections which eventually lead to respiratory failure. Mucins provide protection to epithelia through interaction of their saccharides with bacterial adhesins. Chronic colonization with *Pseudomonas aeruginosa*, is considered the principal cause of death in CF patients. Our laboratory and others have shown that *P. aeruginosa* had considerably stronger binding affinity for CF airway mucin than normal airway mucin. These observations implicate altered glycosylation of CF mucins. Indeed, aberrant glycosylation has been reported for CF mucin. However, to date, the molecular basis of increased interaction between *P. aeruginosa* and CF airway mucin has not been established. We hypothesize that altered glycosylation of CF mucin is responsible for its stronger binding with *P. aeruginosa*. We will determine structural features of the CF mucin carbohydrate ligand(s) that provide increased binding to *P. aeruginosa* by preparing glycopeptides and individual saccharides from CF and control mucins. The glycopeptide(s) which show high inhibition of asialo-GM₁ binding to *P. aeruginosa* will be used to isolate O-linked glycans for further testing of inhibitory activity and structural determination using state-of-the-art highly sensitive mass spectrometry and enzymatic methods. Affinity gels containing selected mucin glycopeptide or mucin saccharide will be used to purify the *P. aeruginosa* adhesins which interact with airway mucins and glycolipids, respectively. The primary structure of the major adhesins will be determined using molecular cloning techniques. Structural characterization of major adhesins will open additional approaches to prevent the binding of *P. aeruginosa* to airway epithelial cells and mucins of CF patients. Information on the adhesin binding sites will permit molecular modeling, design and synthesis of potent O-glycan inhibitors of the *P. aeruginosa* infection. The overall long-term goal of this study is to prevent and/or treat lung infections in CF patients.

Grant: 1R01HL066066-01
Program Director: BANKS-SCHLEGEL, SUSAN P.
Principal Investigator: BAATZ, JOHN E PHD
Title: ROLE OF SURFACTANT PROTEIN B IN INNATE AIRWAY DEFENSE
Institution: MEDICAL UNIVERSITY OF SOUTH CHARLESTON, SC
CAROLINA
Project Period: 2001/07/01-2005/06/30

DESCRIPTION (Applicant's Abstract): Surfactant protein B (SP-B) is essential for postnatal survival and normal surfactant function. SP-B is capable of reorganizing lipid bilayers and is fusogenic protein. Based on these characteristics and the similarity of SP-B's amino acid sequence to those of several antimicrobial peptides, we hypothesized that SP-B itself has antimicrobial properties. Preliminary data obtained by our laboratory have demonstrated he SP-B has in vitro antibacterial activity. The significance of this observation is three-fold. First SP-B is expressed solely in mammalian lungs where it is secreted into the airway lining fluid by bronchial, bronchiolar and alveolar epithelial cells at relatively high concentrations. For that reason, it may act as a component of local mucosal immunity to prevent bacterial infections in these and other regions of the lung. Second, surfactant replacement preparations containing SP-B are presently used for safe treatment of neonatal respiratory distress syndrome, and such preparations do not elicit immunological responses. Third, SP-B is easily isolated or synthesized. Thus, there is potential for safe therapeutic use of SP-B for treatment of pulmonary bacterial infections. Use of SP-B to prevent or eradicate bacterial growth in the airway would be of particular importance in cystic fibrosis (CF), where progressive lung damage occurs as a result of persistent bacterial infection. Moreover, we have found aberrant forms of SP-B in bronchoalveolar lavage (BAL) of adult CF patients and that mature SP-B may be degraded or modified. We will test the hypothesis that SP-B is a component of the innate pulmonary immune system, protecting the human airway against bacterial infection, and this activity may be compromised in the airways of CF patients. The aims of this proposal are 1) to determine molecular forms and activity of SP-B in BAL from CF patients vs. those of normal humans, 2) to delineate the sites in the airway where SP-B is found and in which it may play a defensive role, 3) to characterize the antibacterial activity of SP-B in vitro, and 4) to develop preparations containing native or synthetic SP-B for use as antibacterial agents in vivo. Results from the proposed experiments will lay the foundation for therapeutic use of SP-B for eradication of bacterial lung infections.

Grant: 1R01HL066112-01A1
Program Director: PEAVY, HANNAH H
Principal Investigator: CHAN, EDWARD D MD
Title: Host defense functions of M. tuberculosis lipoglycan
Institution: NATIONAL JEWISH MEDICAL & RES CTR DENVER, CO
Project Period: 2001/07/15-2006/05/31

DESCRIPTION (Adapted from the Applicant's Abstract): Tuberculosis (TB) is the leading cause of death by an infectious agent. Macrophages play a pivotal role in the control of Mycobacterium tuberculosis through the expression of nitric oxide (NO) and TNF α . NO plays an important mycobactericidal role in murine TB and is increasingly recognized to be important in humans. The overall hypothesis of this proposal is that macrophages, by specific surface receptor(s), recognize mycobacterial cell wall products to initiate iNOS- and TNF α -induction. Hence, the focus of this proposal is to: i) determine the receptor and signaling mechanisms which regulate iNOS and TNF α following exposure to the mycobacterial cell wall component lipoarabinomannan (ManLAM) and ii) to determine the significance of each of these components in an in vitro model of infection. Based on experiments showing that macrophages from the Toll-like receptor 4 (TLR4)-mutant C3H/HeJ mice produced significantly lower levels of NO than TLR4-intact C3H/HeN macrophages in response to IFN γ + ManLAM, we hypothesize that ManLAM engages a TLR to induce iNOS-NO*/ TNF α expression. Since non-mannose capped LAM (AraLAM) from M. smegmatis induced greater NO about expression than ManLAM, we hypothesize that ii) the exposed arabinose residues on ManLAM or AraLAM are the components of ManLAM that bind to its putative TLR. Since initial studies show that the mitogen-activated protein kinases (MAPKs) and NF κ B signaling pathways regulate iNOS and TNF α expression, we hypothesize that the proximal kinase MAP/ERK kinase kinase (MEKK) is a pivotal regulator for ManLAM-induction of iNOS and TNF α . Lastly, because TLRs recognize pathogen-derived molecules and enhance host-defenses, we hypothesize that blocking one or more of the TLRs will enhance the growth of M. tuberculosis and inhibit NO* and TNF α expression. These hypotheses will be addressed by three specific aims: 1. To determine the ManLAM structures that mediate the induction of iNOS-NO*/TNF α and the receptor that mediates these ManLAM effects. 2. To investigate the role of the MAPK and NF κ B signaling pathways in ManLAM- and other lipoglycan-induced iNOS-NO* and TNF α . 3. To elucidate the role of the TLRs, MAPK and NF κ B signaling pathways, and ManLAM in controlling the growth of M. tuberculosis in mouse and human macrophages and to correlate effects on growth with NO* and TNF α expression.

Grant: 1R01HL066115-01A1
Program Director: WASSEF, MOMTAZ
Principal Investigator: ROSENFELD, MICHAEL E
Title: C. Pneumoniae and atherosclerotic plaque destabilization
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2001/09/28-2005/06/30

DESCRIPTION (provided by the applicant): Respiratory infection with Chlamydia pneumoniae (C. pneumoniae) is associated with an increased risk of cardiovascular disease morbidity and mortality. C. pneumoniae infection accelerates the development of atherosclerotic lesions in hypercholesterolemic animal models. However, to date it is not known whether C. pneumoniae infection also contributes to the destabilization of established atherosclerotic lesions. Destabilization is characterized by expansion of the necrotic core and erosion of the media and fibrous cap, processes that can lead to plaque rupture with subsequent formation of occlusive thrombi. Cell death, and in particular the death of macrophage-derived foam cells, contributes to the formation and expansion of the necrotic core. It is still unknown precisely what causes the death of cells within atherosclerotic lesions and whether protection from cell death will prevent plaque destabilization. Our preliminary data shows that the combination of prior accumulation of oxidized LDL followed by infection with C. pneumoniae rapidly kills a significant percentage of cultured macrophages. Thus, we hypothesize that 1. recurrent respiratory infection with C. pneumoniae accelerates the destabilization of established atherosclerotic lesions by killing lipid loaded macrophages and smooth muscle cells and 2. inhibition of cell death will reduce plaque destabilization induced by lipid accumulation and C. pneumoniae infection. Our Specific Aims are to 1: Investigate the mechanism(s) by which the combination of lipid loading and infection with C. pneumoniae kills macrophages in vitro. 2: Determine whether repeated infection with C. pneumoniae increases the rate at which lipid loaded arterial macrophages and smooth muscle cells die in vivo and as a result: A) accelerates erosion of established atherosclerotic lesions in the carotid arteries of older apo E knockout mice. B) acutely causes rupture and hemorrhage into established atherosclerotic lesions in the carotid arteries of older apo E knockout mice. 3: Determine whether the overexpression of the anti-apoptotic factor BCL-2 in leukocytes reduces cell death and inhibits: the initiation of fatty streaks and progression of the lesions, plaque destabilization, and the acceleration of plaque destabilization caused by infection with C. pneumoniae in the carotid arteries of apo E knockout mice. These studies may for the first time establish that C. pneumoniae infection accelerates cell death and plaque destabilization and thus help explain why C. pneumoniae infection is associated with an increased risk of mortality from cardiovascular disease.

Grant: 1R01HL066235-01
Program Director: PEAUVY, HANNAH H
Principal Investigator: VOELKER, DENNIS R
Title: SURFACTANT PROTEINS A AND D AND MYCOBACTERIA
Institution: NATIONAL JEWISH MEDICAL & RES CTR DENVER, CO
Project Period: 2000/12/15-2004/11/30

DESCRIPTION (Applicant's Abstract): Pulmonary surfactant is a lipid and protein complex present in the alveolar compartment that resides at the interface between the human lung and the external environment. The constituents of pulmonary surfactant are the first biological components of the host that aerosolized mycobacteria contact upon reaching the alveolar compartment. Surfactant protein A (SP-A) and surfactant protein D (SP-D) are two abundant proteins present in the extracellular fluid of the alveolus that directly interact with mycobacteria and the target cell for replication, the alveolar macrophage. The interactions of mycobacteria with SP-A and SP-D comprise the very earliest events in the process of invasion and infection by the organism. The goals of this project are to understand the physical interactions between SP-A and SP-D and mycobacteria at these very early stages, and determine how this influences the process of bacterial entry and replication within the macrophage. In order to address these goals experimentally, we will use structural mutants of SP-A and SP-D to examine how specific domains of the proteins interact directly with the mycobacteria. The repertoire of mutants we have include point mutations within each of the four major structural domains, and chimeric proteins between SP-A and SP-D and Mannose Binding Protein A. These studies will enable us to determine which protein domains are required for mycobacterial recognition. We will also determine the role of SP-A and SP-D in promoting the successful entry of mycobacteria into the macrophage for replication. The SP-A and SP-D structural variants will be used to map which protein domains are required for the interactions with the macrophage that promote mycobacterial infection. In order to understand the interactions of the mycobacteria with SP-D in more detail, we will prepare derivatives of a major mycobacterial membrane envelope component, mannosyl-lipoarabinomannan, and cocrystallize them with a truncated version of the human SP-D molecule. These latter studies will enable us to define at the atomic level how the mycobacterial envelope interacts with the surfactant proteins. Collectively, the proposed studies will provide us with a detailed map of interactive sites between the surfactant proteins and mycobacteria and macrophages that will create new opportunities for developing agents that interfere with the infection process.

Grant: 1R01HL066415-01
Program Director: BANKS-SCHLEGEL, SUSAN P.
Principal Investigator: BELAAOUAJ, ABDERRAZZAQ PHD
Title: SERINE PROTEINASES & LUNG HOST DEFENSE AGAINST BACTERIA
Institution: BARNES-JEWISH HOSPITAL ST. LOUIS, MO
Project Period: 2001/02/15-2005/01/31

DESCRIPTION (Applicant's abstract): Human and mouse neutrophils contain neutrophil elastase (NE), cathepsin G (CG) and proteinase 3 (PR 3). The capacity of these serine proteinases, especially NE, to kill bacteria in vitro and to cleave extracellular matrix (ECM) proteins leading to tissue damage is well established, but whether these enzymes kill bacteria in vivo and have specific bacterial target molecules remains unknown. Also, the potential role of the ECM degradation products generated by these proteinases in host defense against bacteria has not been explored. Using mice deficient in NE and CG, we have demonstrated that NE, but not CG, reduces mortality from Gram negative bacterial infections. To date, the antibacterial role of PR 3 has not been clarified in vivo. We propose to generate mice deficient in PR 3 by gene targeting and subject them to bacterial lung infections to determine the relative importance of PR 3 in host defense against bacteria in the lung. Our preliminary data show that the outer membrane protein (Omp) A represents a critical target for NE-mediated killing of E. coli. We hypothesize that Omps represent essential targets of NE to kill other Gram negative bacteria. Recently, we have found that NE kills *Pseudomonas aeruginosa* in vitro, and degrades its major Omp F, which is unrelated to Omp A. We propose to determine the importance of Omp F in NE-mediated killing of *Pseudomonas aeruginosa* in isolated neutrophils and in vivo models of acute and chronic pulmonary infections. Wild type *Pseudomonas aeruginosa* and isogenic strains deficient in Omp F will be used in these studies. We have observed that elastin peptides from NE-digested human lung elastin are bactericidal for *Klebsiella pneumoniae* in vitro. These data constitute the first evidence of antibacterial role of ECM derived peptides and reveal a novel role for ECM. We will isolate these elastin fragment(s) and investigate their bactericidal activity in vitro and in vivo. These studies will advance our knowledge of the functional properties of lung serine proteinases and ECM peptides. Also, characterization of NE-degraded elastin may provide novel antimicrobial peptides in humans.

Grant: 1R01HL066436-01
Program Director: TOLUNAY, ESER
Principal Investigator: ARDITI, MOSHE MD
Title: MOLECULAR PATHOGENESIS OF CHLAMYDIA-INDUCED ATHEROGENESIS
Institution: CEDARS-SINAI MEDICAL CENTER LOS ANGELES, CA
Project Period: 2001/04/01-2005/03/31

DESCRIPTION (Adapted from the Applicant's Abstract): The goal is to define mechanisms leading to the development and progression of chlamydiae-mediated atherosclerosis. Approaches will center on studying signaling mechanisms for cLPS and cHsp60, effector molecules known to activate host pro-inflammatory pathways. The hypothesis to be investigated is that cLPS and cHsp60 utilize the Toll-like Receptor-4 (TLR-4) to activate macrophages and endothelial cells via NF- κ B and MAPK through myeloid differentiation protein (MyD88). The effects of this activation will lead to increased expression of pro-inflammatory cytokines (IL-6, IL-8), adhesion molecules (ICAM-1 VCAM-1), growth factors (M-CSF), cyclooxygenase-2 (Cox-2) and enhanced transendothelial cell migration of monocytes; all factors implicated in atherogenesis. The hypothesis also will be tested in vivo by determining if apo-E^{-/-}, TLR-4^{-/-} double knockout mice remain indifferent to *C. pneumoniae*-induced accelerated lesion progression. The specific aims are to (1) determine if TLR-4 is the signaling receptor for cLPS and cHsp60 and to investigate the signaling pathways induced (ERK1/ERK2, p38MAPK and JNK) and other downstream events leading to NF- κ B activation; (2) to define the molecular mechanisms involved in cLPS and cHsp60-induced transendothelial cell migration of monocytes; and (3) to create double knockout mutants in mice (TLR4^{-/-}, apoE^{-/-} and MyD88^{-/-}, apoE^{-/-}) to investigate the contributions of TLR-4 and MyD88 in the initiation and progression of atherosclerosis in the presence and absence of *C. pneumoniae* infection. The results of these studies are expected to lead to new targets for intervention and prevention of coronary atherosclerosis.

Grant: 1R01HL066453-01
Program Director: GANGULY, PANKAJ
Principal Investigator: ROSS, JULIA M BS
Title: DYNAMIC PLATELET - STAPHYLOCOCCAL INTERACTIONS
Institution: UNIVERSITY OF MARYLAND BALT CO BALTIMORE, MD
CAMPUS
Project Period: 2001/09/01-2005/08/31

DESCRIPTION (Verbatim from Applicant's Abstract): The broad objective of the proposed research is to comprehensively characterize the molecular interactions between *Staphylococcus aureus* and platelets as a function of the dynamic shear environment in order to provide a rational basis for the development of novel treatments to combat staphylococcal cardiovascular infections. The hypothesis to be tested is that shear stress affects the adhesive interactions between platelets and *S. aureus* by modulating the (i) relative importance of the adhesive molecules involved and (ii) the reaction binding kinetics. The proposed approach uses controlled, dynamic, in vitro experimental systems to systematically and comprehensively examine the importance of platelet activation, blood components, blood flow, and bacteria in the development of blood-born staphylococcal infections. A long-term goal of this work is to investigate the interrelationship between thrombogenesis and cardiovascular infection mechanisms. The specific aims of the project are to: 1) comprehensively elucidate the molecular mechanisms of *S. aureus*-platelet interactions under shear conditions of direct physiological relevance; 2) characterize *S. aureus*-platelet heteroaggregation in cell suspensions subjected to controlled levels of shear and; 3) develop a protocol to study *S. aureus*-platelet aggregation in whole blood and to evaluate the effect of this extension on *S. aureus*-platelet interactions under shear conditions. Completion of these specific aims will provide a rational basis for the design of new therapeutic molecules to block specific adhesion events, as well as identify the most important bacterial receptors to target in vaccine development.

Grant: 1R01HL068127-01
Program Director: MUSSON, ROBERT A.
Principal Investigator: CHRONEOS, ZISSIS C PHD
Title: SP-A Receptor Modulation of Macrophage Phagocytosis
Institution: UNIVERSITY OF TEXAS HLTH CTR AT TYLER, TX
TYLER
Project Period: 2001/07/01-2005/05/31

Pulmonary mycobacterial infections affect approximately one third of the world's population and claim millions of lives annually. Infectious organisms that are inhaled into the airspaces encounter alveolar macrophages in the context of immune proteins in the alveolar lining fluid. Specifically, surfactant protein A (SP-A), a lung- specific collectin, orchestrates macrophage activation, phagocytosis and killing of mycobacteria via a 210 kDa (SP-8210) receptor. The long term objective of this application is to elucidate the mechanisms by which SP-A and its receptor direct alveolar macrophage host defense functions in the lung *in vivo*. This proposal is based on these new findings: 1) the SP-A receptor is a heterooligomer of 210 kDa (SP-8210) and 240 kDa (SP-8240) cell- surface, and 78 kDa (SP-R78) intracellular proteins. Both SP-8210 and SP-R78 have been sequenced by mass spectrometry. 2) The composition of the SP-A receptor heterooligomer may vary based on the state of macrophage differentiation. The central hypothesis of this proposal is that the interaction of SP-A with its receptor coordinates macrophage activation and mycobacterial clearance via distinct SP-A receptor- directed mechanisms. To test this hypothesis we will study SP-A receptor structure and function in the context of mycobacterial infection. The Specific Aims are: 1) reconstitute a functional SP-A receptor in COS .cells and use a panel of recombinant wild type and mutant SP-A proteins to determine mechanisms of SP-A- binding and function in the phagocytosis of mycobacteria, 2) determine the role of the intracellular SP-R78 in the activation of an SP-A-specific pathway for intracellular targeting of mycobacteria, and 3) study the relative expression of the SP- A receptor components during macrophage differentiation to understand how the structure of the SP-A receptor directs SP-A- mediated mycobacterial clearance and activation of macrophages. To facilitate these studies we will utilize immature alveolar macrophages that we have isolated, and *Mycobacterium bovis* BCG and *Mycobacterium tuberculosis* Ra reporter strains that express green fluorescent protein.

Grant: 1R01HL068513-01
Program Director: PEAUVY, HANNAH H
Principal Investigator: SMITH, ISSAR PHD BIOLOGY NEC:BIOL
NEC-UNSPEC
Title: M. tuberculosis & host gene expression during infection
Institution: PUBLIC HEALTH RESEARCH INSTITUTE NEW YORK, NY
Project Period: 2001/09/10-2006/07/31

DESCRIPTION (provided by applicant) The sequencing of many genomes has led to the development of new technologies for studying gene expression in prokaryotic and eukaryotic cells. These techniques, especially gene expression profiling with DNA arrays are revolutionizing studies of microbial physiology, eg, cellular responses to different growth conditions and environmental stimuli, etc. These new methods can also be used to study bacterial and/or host gene expression during infections, as they facilitate transcriptional analyses of the interaction between bacterial pathogens and their host. The purpose of this grant application is to do an integrated study of bacterial and host gene expression during M. tuberculosis infection of macrophages and animals, using standard DNA array technology and by developing new methods, as well. The aims of the project are as follows: 1) Expression profiling of M. tuberculosis genes in vitro and during infection. We will use DNA arrays containing the entire M. tuberculosis 4000 gene set (Mtb gene array) to study the levels of all the mRNAs of wild type and mutant M. tuberculosis in vitro in response to various environmental stresses. The arrays will also be used to study the levels of mRNAs of wild type and mutant M. tuberculosis during infection of human and mouse macrophage cell lines. We will also develop molecular beacon derived methods described in specific aim 3 to study the expression of M. tuberculosis genes in different tissues during the infection of animals. 2) the second major aim is to do expression profiling of host genes during M. tuberculosis infections. For these studies, we will use Affymetrix or custom arrays of the human and mouse genomes to study host gene expression during M. tuberculosis infection of macrophages and during later stages of infection in different tissues of infected animals. 3) the final aim is to develop new methods for studying M. tuberculosis gene expression during animal infections. These methods will use molecular beacons and related techniques for fixed arrays that will allow the detection and quantitation of multiple M. tuberculosis mRNAs during infection and will employ specific amplification during hybridization so that low levels of bacterial mRNAs can be specifically detected in the presence of high levels of host RNA.

Grant: 1R01HL068517-01
Program Director: PEAUV, HANNAH H
Principal Investigator: PINE, RICHARD I PHD
Title: Functional Genomics of Interferon for Tuberculosis
Institution: PUBLIC HEALTH RESEARCH INSTITUTE NEW YORK, NY
Project Period: 2001/09/10-2006/07/31

DESCRIPTION Interferon-gamma (IFNgamma) is a cytokine released by TH1 lymphocytes in tuberculosis and is an essential mediator of the cellular immune response, producing macrophage activation. Signaling is transduced through activation of STAT-1, a latent transcription factor, and leads to induced expression of other transcription factors. STAT-1 and the other transcription factors like IRF-1 together increase expression of effector genes such as iNOS. We have been conducting a clinical trial of aerosolized IFNgamma as treatment for pulmonary tuberculosis and have reported that patients with multi-drug resistant tuberculosis improved on this regimen. We now report that aerosolized IFNgamma induces STAT-1 and IRF transcription factors in lung cells. Treatment also markedly reduces HIV-1 levels in the lungs of AIDS patients with tuberculosis, providing further evidence of enhanced lung immunity produced by pharmacological doses of IFNgamma. THP-1 macrophages and alveolar macrophages are similar with regard to induction of STAT-1, IRF-1 or IRF-9 (also called p48 and ISGF3-gamma) after infection with *M. tuberculosis* (*M.tb*) or IFNgamma treatment. THP-1 macrophages respond to IFNgamma with increased IRF-9 mRNA but do not change its transcription, demonstrating that IRF-9 is up-regulated by post-transcriptional mechanisms such as splicing or message stability. We used functional genomics to identify other IFNgamma responsive genes. Of 4375 genes present on a high density filter array, 48 were induced and 13 were repressed after 4 hours of IFNgamma stimulation. Only 15 of these 61 genes have been reported to be responsive to IFNgamma. Finally, with the goal of assaying genome wide transcription rates, we have developed a novel assay for transcription using Br-UTP labeled nascent RNA. Our goal is to identify genes unique to the cellular immune response during tuberculosis in vivo and genes induced or repressed by IFNgamma and/or *M.tb*. ex vivo and in vivo. We propose to use functional genomics to identify the genes induced and repressed in: 1) THP-1 macrophages infected with *M.tb* and/or treated with IFN-gamma in vitro 2) Alveolar macrophages infected with *M.tb* and/or treated with IFNgamma ex vivo and 3) Bronchoalveolar lavage cells from tuberculosis patients before and after aerosolized IFNgamma. We will then assay transcription rates of the genes that are IFN responsive to define the contribution of transcriptional and post transcriptional regulation to mRNA abundance. We hypothesize that like IRF-9, other genes important to the IFNgamma response are post-transcriptionally regulated in macrophages. This analysis will further the understanding of cellular immunity in tuberculosis and gene expression regulated by IFNgamma.

Grant: 1R01HL068520-01
Program Director: PEAVY, HANNAH H
Principal Investigator: JAGANNATH, CHINNASWAMY
Title: Reactivation Tuberculosis in A/J Mice
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX
HOUSTON
Project Period: 2001/09/30-2006/07/31

DESCRIPTION (provided by applicant) One quarter of the world's population is infected with *M. tuberculosis* resulting in approximately 2.9 million deaths each year. Reactivation tuberculosis is the major cause of adult tuberculosis today although the mechanisms that predispose to reactivation are complex and enigmatic. Since animal models are useful for understanding pathogenesis of tuberculosis, we performed a series of studies to standardize murine models of acute, chronic and reactivation tuberculosis by varying the mouse strain, route and dose of infection. We then found that A/J mice were highly susceptible while C57Bl/6 mice were relatively resistant to progressive disease when infected either i.v. or via aerosol routes. A/J mice were unable to form granulomas in lungs and their macrophages were defective in killing MTB. Unlike C57Bl/6 mice, A/J mice also underwent an early and near uniform reactivation of tuberculosis following the Cornell model. A/J mice have a deletion in the gene encoding for Complement C5 which in intact mice yields the C5a anaphylatoxin, a known regulator of cytokine and chemokine synthesis of macrophages. Therefore, in this investigation, we will examine the hypothesis that the lack of C5a compromises the immune responses in mice allowing the reactivation of tuberculosis through the following aims. Specific Aim I will investigate whether the deletion in C5 gene affects the synthesis of cytokines (TNF alpha, IL1-beta and IL-6), prevents macrophage activation and thereby macrophage mediated killing of MTB in A/J mice. Specific Aim II will investigate the effects of the deletion in C5 gene to the secretion of chemokines by MTB infected A/J macrophages and evaluate whether they are important in causing influx of immune cells into the lungs and formation of granulomas. Specific Aim III will characterize histological, cytokine and chemokine responses of lungs in A/J mice to determine the type of immune response (Th1 vs Th2) that dominates during the reactivation of tuberculosis. These studies are anticipated to enhance our understanding on putative mechanisms that precede the reactivation of tuberculosis in the lungs of mice and ultimately help us to develop better strategies to prevent tuberculosis in man.

Grant: 1R01HL068525-01
Program Director: PEAVY, HANNAH H
Principal Investigator: EHRT, SABINE PHD
Title: Macrophage Gene Expression: Impact of M. tuberculosis
Institution: WEILL MEDICAL COLLEGE OF CORNELL NEW YORK, NY
UNIV
Project Period: 2001/09/10-2006/07/31

DESCRIPTION (provided by applicant) One third of the world's population is currently infected with Mycobacterium tuberculosis (Mtb) and every second, another person is newly infected with Mtb around the world. Each year an estimated 8 million people develop clinical disease and 1.87 million people die of TB. The primary host cell of Mtb is the macrophage and the Mtb-macrophage interaction is critical to every phase of Mtb's infectious cycle. Gene expression analysis (GEA) is the most effective technology currently available to record a quantitative picture of a cell's functional state, and therefore to compare cell states and types. The critical need for such a comparison in TB research is heightened when one takes into account the heterogeneity of macrophages by organ, species of origin and donor history. The goals of this study are to extend our understanding of macrophage biology relevant to tuberculosis (TB), and in so doing, to define the extent to which mouse macrophages can serve as surrogates for human macrophages. The latter question is key if functional genomics is used as a tool in TB research to test the course of infection by wild type and genetically modified Mtb in wild type and genetically modified mice. Specifically, we will extend our current use of high-density oligonucleotide microarrays, quantitative PCR and in situ hybridization (ISH) to analyze and compare the gene expression profiles of three distinct populations of primary cells: mouse bone marrow macrophages (BMM), mouse pulmonary alveolar macrophages (PAM), and human PAM. These relatively pure macrophage populations will be compared with the heterogeneous mixture of all cells in mouse lung. Both human and mouse cells will come both from normal donors and individuals with active TB. Each cell population will be studied without further treatment, after infection in vitro with virulent Mtb, and/or after exposure to interferon gamma (IFNgamma), a cytokine critical for control of mycobacterial infection in both humans and mice. These comparisons will allow us to validate or qualify the use of mouse PAM, mouse BMM and human PAM as model systems for studying macrophage-Mtb interactions. The identification of genes regulated by Mtb in macrophages will generate hypotheses with respect to the role of these genes in pathogenesis. We will test at least one such hypothesis, that secretory leukocyte protease inhibitor (SLPI) plays a role in the pathogenesis of TB. Finally, we will share our GEA with others via the internet so that as many of the resulting hypotheses as possible can be rapidly and independently explored.

Grant: 1R01HL068526-01
Program Director: PEAVY, HANNAH H
Principal Investigator: KIRSCHNER, DENISE E PHD
Title: The dynamics of granuloma formation in tuberculosis
Institution: UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI
Project Period: 2001/09/30-2006/07/31

DESCRIPTION (provided by applicant) The goal of this proposal is to explain the formation of granuloma in infection with Mycobacterium tuberculosis. Understanding granuloma formation and function will elucidate the primary immune mechanism for controlling tuberculosis infection. Our goal is to simulate the process of granuloma formation on a spatio-temporal scale and present the results in a time-lapse movie format. This will yield an interactive tool to study the role of specific immune elements in granuloma formation and function. Development of a virtual model of human infection will allow for integration of the plethora of chemokine, cytokine, cellular influx information and other relevant immunological factors, as generated by experimental systems. To this end, powerful techniques (e.g., microarrays) are available for obtaining comprehensive gene expression data. Using these methods to survey expression within the granulomas of non-human primates and mice will enable us to determine which immunological mediators are involved in granuloma formation, what the timing of their expression is in the formation, and their location within the granuloma. Further studies will indicate which cell-types are expressing which mediators. Our specific aims are to: (1) Identify the temporal and spatial expression of host immune elements participating in granuloma formation using gene expression tools in murine models of tuberculosis (2) Identify the temporal and spatial expression of host immune elements participating in granuloma formation using gene expression tools in murine models of tuberculosis (3) Determine the dynamics of granuloma formation and function in humans using mathematical models of the granuloma response in tuberculosis. Through this unique approach, the interaction of multiple factors that control the formation of the granuloma will be defined. Key parameters governing these interactions will be identified. The ability to synthesize the data generated by the experiments in the models allows for an understanding of the dynamics of granuloma formation as more than the sum of its parts.

Grant: 1R01HL068532-01
Program Director: PEAUVY, HANNAH H
Principal Investigator: APT, ALEXANDER S PHD
Title: Identification of genes controlling TB in murine lungs
Institution: CENTRAL INSTITUTE FOR TUBERCULOSIS MOSCOW,
Project Period: 2001/09/30-2006/07/31

DESCRIPTION (provided by applicant) Elucidation of the mechanisms of host resistance against Mycobacterium tuberculosis infection and of TB pathogenesis is a high objective. Identification of genes and their alleles that confer resistance versus susceptibility to TB provides deep insight into basic mechanisms of immunity and pathology. Limitations to identifying human TB susceptibility genes are the polygenic control of susceptibility and the absence of clearly delineated phenotypes required for genetic analysis. Animal models of TB proved to be extremely valuable in elucidating immunity to mycobacteria and genetic control of susceptibility/resistance. Recently we and others accomplished a provisional genome-wide linkage analysis of TB susceptibility in mice, and mapped several quantitative trait loci (QTLs) in the genome control the course of the disease. Despite the fact that chromosome regions surrounding all QTLs contain genes that regulate the function of cells of the immune system (candidate genes), the physiologic basis for the difference in susceptibility to TB remains unknown and is a subject of this research project. To determine the genes that are differentially expressed in lung macrophages of susceptible and resistant mice following mycobacterial infection we have established a culture model of lung macrophages infected with mycobacteria that exactly follows genetic pattern of TB susceptibility control. We propose to compare gene expression in normal and M. tuberculosis-infected macrophages from susceptible and resistant mice using a DNA chip technology that allows the monitoring of more than 11,000 mouse genes simultaneously. To genetically dissect susceptibility to TB at the organism level, we will establish two independent pairs of congenic mouse strains. In each pair, genetic difference will be restricted to a small chromosome segment surrounding a particular QTL, one on distal chromosome 3 and the other on proximal chromosome 9. We will study gene expression in macrophages in these novel mouse strains and thus link shifts in gene expression with the alleles of particular QTLs. We will perform a new genome screening experiments, employing combination of strain in which an unusual inheritance of resistance with the strong heterosis effect was observed, in order to identify the novel chromosomal regions participating in TB control. We will define the chromosome 3 and 9 QTLs map location to approximately 1 cM intervals by a sequential 2-stage interval-specific congenic strains approach, and we will clone corresponding QTLs relying on testing of candidate genes available from complete gene map of the mouse.

Grant: 1R01HL068533-01
Program Director: PEAVY, HANNAH H
Principal Investigator: SCHURR, ERWIN PHD
Title: GENETIC DISSECTION OF MYCOBACTERIAL INFECTION
Institution: MC GILL UNIVERSITY MONTREAL, PQ
Project Period: 2001/09/14-2006/07/31

DESCRIPTION (provided by applicant) The objective of the studies proposed in this application is to identify genotypic combinations of mice and mycobacteria that result in significant alterations of host responses to experimental mycobacterial infection. Susceptibility or resistance to experimental infection will be defined by determination of median survival time, weight loss, mycobacterial load in the lung and spleen, and in addition various immunological parameters in lung and other tissues. In these experiments, the host genome will be varied by use of 37 recombinant congenic strains (RCS), which are derived from inbred progenitors that are either susceptible to tuberculosis (A/J, abbreviated A) or resistant to tuberculosis (C57BL/6J, abbreviated B). The AcB/BcA RCS are now fully inbred and genotyped with a dense set of genome-wide microsatellite markers. These strains will be infected with a panel of *Mycobacterium tuberculosis* and *M. bovis* strains that have been deleted for genome regions associated with attenuation of BCG vaccines. The infection protocol in the RCS will reveal informative, significant deviations from the "expected" or "parental" disease phenotypes which signify the presence of quantitative trait loci (QTL) with strong effect on phenotype expression and possibly specific gene(s) interaction between the host and pathogen. In RCS carrying QTL with strong effect on phenotype expression, changes in gene expression level in both lung macrophages and intracellular mycobacteria will be revealed by microarray analysis. The knowledge to what extent host responses in mycobacterial infections are a reflection of specific host pathogen combinations will be crucial for our understanding of the epidemiological flow of *M. tuberculosis* through an exposed population.

Grant: 1R01HL068534-01
Program Director: PEAUVY, HANNAH H
Principal Investigator: SCOTT, WILLIAM K PHD
Title: Genetic Studies of Human Susceptibility to Tuberculosis
Institution: DUKE UNIVERSITY DURHAM, NC
Project Period: 2001/09/10-2006/07/31

DESCRIPTION (provided by applicant) Tuberculosis (TB) is currently and historically an enormous public health problem. Approximately one-third of the world's population are currently infected with *Mycobacterium tuberculosis* (*M. tuberculosis*) and TB accounts for over 25% of preventable adult deaths world-wide. Despite the high infection rate, only about 10% of people infected with *M.tb* ever become sick with active TB. Evidence suggests that progression to active TB is influenced by host genetic factors. For example, the epidemiology of TB suggests that genetic selection takes place after introduction of *M. tuberculosis* to the population; genetically susceptible individuals succumb to the infection and relatively resistant individuals survive to reproduce. As well, twin studies demonstrate higher concordance rates for TB among identical twins, compared to fraternal twins. Mouse models of mycobacterial infection have identified several potential susceptibility loci, such as the gene named *Nramp1*, as well as several cytokine and cytokine receptor genes. Family-based linkage studies and case-control studies of candidate genes in humans suggest roles for these and other genes associated with development of TB in humans. In light of these observations, we propose a family-based association study of candidate genes for TB susceptibility. To accomplish the goal of identifying genes influencing susceptibility to TB we specifically propose to: 1) Ascertain 1,000 parent- child triads (500 Caucasian, 500 African-American) from North and South Carolina for genetic studies of TB susceptibility genes. 2) Test candidate genes in the first 500 parent-child triads. Multiple single nucleotide polymorphisms (SNPs) will be genotyped in each gene and analyzed using family-based tests of association; significant results will be followed-up in the remaining 500 triads. 3) Examine the relationship between candidate genes and other clinical variables such as PPD skin test results, disease severity, treatment relapse and failure, and presence of extrapulmonary disease. 4) Evaluate gene-gene and gene-environment interactions using multivariable models and data reduction techniques such as the multifactor dimensionality reduction (MDR) method.

Grant: 1R01HL068537-01
Program Director: PEAVY, HANNAH H
Principal Investigator: HUNTER, ROBERT L
Title: Genetic Control of Pulmonary Cavities in Tuberculosis
Institution: UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX
HOUSTON
Project Period: 2001/09/10-2006/07/31

DESCRIPTION (provided by applicant) Since it has no animal or environmental reservoir, M. tuberculosis (MTB) must be aerosolized by diseased individuals in order to propagate. The organism accomplishes this most effectively by forming pulmonary cavities. One of us (PMS) has recently observed genetic deficiencies in MTB strains with reduced capacity to cause pulmonary cavities in humans. Another of us (RH) has developed a mouse model that suggests the toxicity of cord factor (trehalose 6,6" dimycolate (TDM)) contributes to the formation of cavities. Together, we now propose to identify and characterize MTB genes that control the formation of pulmonary cavities. We will use: 1) two different DNA microarrays to determine, on a genome wide scale, the presence and expression profile of all MTB genes; 2) a new mouse model that manifests the type of caseating granuloma that gives rise to cavities; and 3) conventional genetic approaches for gene knock-outs and complementation. We will build a matrix database of well characterized clinical isolates that records clinical cavity formation, results of assays of toxic lipids and whole genome DNA microarray identification of gene deletion and expression. Analysis of this database will generate specific hypotheses as to which mycobacterial genes are associated with the production of toxic lipids and/or are responsible for pulmonary cavities. These hypotheses will be tested using conventional genetic approaches and by further application of our mouse model. Specifically, we will use three existing NIH funded collections to identify 30 mycobacterial clones that do and 30 that do not cause cavitation. We will grow these isolates as pellicles and assay the amount, distribution, structure and toxicity of TDM. Next, we will use a Bayesian statistical approach to identify genes whose presence or expression is associated with loss of cavity production in humans and/or with the production of toxic TDM. In parallel, we will validate the relevance of our animal model using strains that do or do not produce cavities in humans. Finally, we will use conventional genetic approaches to knock out or insert genes associated with cavity formation and will test the capacity of these modified strains to produce TDM (or other relevant parameters) and induce cavities in our mouse model. If successful, new insights into the pathogenesis of pulmonary cavities may lead to specific interventions to prevent this highly infectious disease manifestation.

Grant: 1R01HL068543-01
Program Director: PEAVY, HANNAH H
Principal Investigator: ROMAN, JESSE R MD BIOLOGY NEC:BIOLOG
NEC-UNSPEC
Title: Regulation of host response genes in pathogenesis of TB
Institution: EMORY UNIVERSITY ATLANTA, GA
Project Period: 2001/09/10-2006/07/31

DESCRIPTION (provided by applicant) Mycobacterium tuberculosis (Mtb) is the leading infectious cause of death worldwide. Our inability to control the spread of this disease and the absence of new effective chemotherapeutic agents are due in part to the limited knowledge about host genes that control granuloma formation and other aspects of the host's response to this pathogen in lung. One host response considered important in tuberculosis is that of tissue remodeling which is characterized by alterations in extracellular matrix expression and degradation. Tissue remodeling is responsible for the development of Mtb- mediated fibrosis, bronchiectasis, and cavitation. Although these processes are often considered late manifestations of pulmonary tuberculosis, mounting evidence suggests that the genes involved in the control of tissue remodeling (TR genes) are expressed very early in lung after Mtb infection, and are involved in other key processes including leukocyte recruitment and granuloma formation. Consistent with this, we have demonstrated that: 1) Mtb Erdman bacilli and isolated cell wall components of Mtb induce the expression of TR genes encoding for extracellular matrices, matrix-degrading proteases, and pro-fibrotic growth factors in vitro (i.e., human monocyte/macrophages) and in vivo (C57BL/6 mice). In mice, the induction of TR genes correlated both spatially and temporally with the inflammatory response. 2) The induction of TR genes in monocyte/macrophages by Mtb occurs via receptor-mediated protein kinase pathways and requires the induction of specific transcription factors (e.g., AP-1). 3) The injection of trehalose-6,6'-dimycolate (previously called mycobacterial cord factor) or live Mtb Erdman strain into mice with knockout mutations in a TR gene (Matrix Metalloproteinase-9) resulted in increased inflammation and granuloma formation. Together, this information suggests that the interaction between Mtb and host cells triggers TR gene expression; in turn, the products of TR genes play important roles in the host response to Mtb. The overall goal of this application is to identify the TR genes that are differentially expressed in pulmonary tuberculosis and study their function. This will be accomplished in 3 specific aims designed to: 1) Identify host TR genes differentially expressed in vitro in human monocyte/macrophages after infection with Mtb using High Density Oligonucleotide Array or HDOA. 2) Identify the TR genes differentially expressed in the lungs of infected mice and in the lungs of humans with pulmonary tuberculosis using HDOA. 3) Determine the function of specific TR genes identified in Specific Aims I and II by infection of mice with gene knockout mutations. We propose to study the function of 3 (at the most 4) TR genes. We will begin exploring the function of 2 TR genes encoding for matrix metalloproteinases which we have demonstrated to be differentially expressed in Mtb-infected lungs and for which knockout animals are already available in our laboratory.

Grant: 1R01HL069114-01A1
Program Director: THOMAS, JOHN
Principal Investigator: STYLES, LORI A MD
Title: Pediatrics:Chlamydia, Sickle Cell Anemia and Stroke Risk
Institution: CHILDREN'S HOSPITAL & RES CTR AT OAKLAND, CA
OAKLAND
Project Period: 2001/09/24-2005/06/30

DESCRIPTION (provided by applicant): Infection with Chlamydia pneumoniae (C. pneumoniae) is associated with an increased risk of cerebrovascular disease in the general population. Children with sickle cell anemia (SCA) are 200 times more likely to have cerebrovascular disease than normal children and are known to have an altered immune response to many infectious pathogens. C. pneumoniae is the leading infectious cause of acute chest syndrome which, interestingly, is a well- established risk factor for stroke in children with SCA. Our preliminary data indicates that SCA patients with MRI-documented cerebral infarction are 12 times more likely to have C. pneumoniae infection than SCA patients with normal MRI scans. We hypothesize that SCA patients have an abnormal immune response to C. pneumoniae that results in persistent infection which, in turn, triggers the development of cerebrovascular disease. Sickel cell anemia patients with an elevated velocity on transcranial doppler ultrasound (TCD) are known to be at high risk to develop stroke and an elevated TCD likely reflects underlying vascular disease. In addition, the Stroke Prevention in Sickel Cell Anemia Trial (STOP) demonstrated that almost 40% of children with an elevated TCD have evidence of cerebral infarction on MRI. Children with abnormal TCDs are, therefore, an appropriated population to investigate an association between cerebrovascular disease and C. pneumoniae infection. As an ancillary study of the STOP II trial we propose 1) To determine if C. pneumoniae infection is associated with cerebral infarction in children with SCA; 2) To characterize the immunological response to C. pneumoniae infection in patients with SCA. Establishing a link between C. pneumoniae infection and cerebral infarction will open the door to novel, less toxic approaches to the treatment and prevention of stroke in SCA, including antibiotics and vaccines. The data gained in this proposal would provide the preliminary data necessary to justify further clinical trials.

Grant: 1R01HL069116-01
Program Director: NOEL, PATRICIA
Principal Investigator: BUSSE, WILLIAM W
Title: Severe Asthma From Respiratory Infections
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 2001/09/20-2006/06/30

DESCRIPTION (provided by applicant): Chronic severe asthma affects only a subsegment of patients with asthma and is characterized, in part, by persistent airflow obstruction and symptoms despite ongoing treatment. Because of the severity of their disease, this population of asthmatic patients has the greatest morbidity and health care costs. At present, the mechanisms that cause severe asthma are not fully established. The overall goal of this research project is to define the features of severe asthma, identify the mechanisms that lead to this phenotype and discover possible inroads for new and more effective therapies. Based upon existing evidence and preliminary data, it is the hypothesis of this research project that severe asthma is caused, in some patients, by a persistent respiratory infection by viruses (i.e. rhinovirus, respiratory syncytial virus, or adenovirus), Mycoplasma pneumonia, or Chlamydia pneumonia. It is further proposed that these agents infect lower airway epithelium and macrophages to enhance the production of inflammatory cytokines/chemokines (i.e. IL-8) and recruitment of inflammatory cells, particularly neutrophils, to further airway injury and airflow obstruction. To accomplish these goals, subjects with severe asthma (i.e. FEV1 <75% predicted, and ongoing symptoms despite high doses of inhaled corticosteroids) will be recruited along with three groups for comparison: Mild asthma, asthma with airflow obstruction, i.e. FEV1 <75% predicted, but reversible to beta agonists, and normals. Measurements of preliminary physiology will be made in these four groups to test the hypothesis that severe asthma is characterized by airway-parenchymal uncoupling. In addition, imaging techniques will be used to correlate determinants of pulmonary physiology with airway structure, by high resolution computerized tomography, and ventilation abnormalities, by magnetic resonance imaging with inhaled hyperpolarized helium. To determine the characteristics of inflammation in peripheral blood, sputum, and lavage fluid, mucosal biopsies will be obtained to assess the hypothesis that neutrophilic inflammation and IL-8 are characteristic features of fixed airway obstruction in severe asthma whereas eosinophilic injury and IL-5 are associated more with reversible airway obstruction. Finally, PCR and immunohistochemistry of lavage fluid and mucosal biopsies will be used to test the hypothesis that severe asthma and fixed airway obstruction with IL-8 and neutrophils are associated with a persistent respiratory infection by respiratory viruses (i.e. adenovirus, influenza, respiratory syncytial virus or rhinovirus), Mycoplasma pneumonia, or Chlamydia pneumonia. It is proposed that these studies will provide new insights into mechanisms of asthma, and particularly severe persistent disease, and potential new approaches to treatment.

Grant: 1R01HL069425-01
Program Director: MUSSON, ROBERT A.
Principal Investigator: PAN, ZHIXING K MD
Title: LPS signaling and Lung Innate Immunity
Institution: SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA
Project Period: 2001/09/30-2005/07/31

DESCRIPTION (provided by applicant): Exposure to lipopolysaccharide (LPS) induces an inflammatory reaction in the lung mediated primarily by blood monocytes and alveolar macrophages, which release an array of inflammatory cytokines including IL-8, TNF, IL-1 and IL-6. Interestingly, the ability of the host to respond to LPS is highly variable. Differences between individuals have been reported in the release and synthesis of cytokines by human monocytes stimulated with LPS. Therefore, elucidating the mechanisms that regulate LPS-mediated gene transcription in alveolar macrophages is crucial for understanding the pathogenesis of lung inflammatory disease and the interactions between lung innate immunity and inflammatory responses. We've also demonstrated that LPS stimulated IL-8 expression in alveolar macrophages involves activation of the PI3K, Rho family of small G-proteins, with subsequent activation of NF- B. The primary hypothesis to be tested is that the observed clinical variability in LPS-induced lung inflammation is directly related to differences in LPS-stimulated gene transcription in alveolar macrophages. We further propose that these differences are determined by the signaling cascade linking TLR4 to activation of NF- B. Three broad approaches will be utilized in this proposal. First, we have developed a compelling body of preliminary data suggesting that LPS-induced NF- B activation and IL-8 expression in alveolar macrophages requires Rho GTPase activity. This proposal will therefore elucidate the molecular details of the role of the Rho family of GTPases in LPS-induced NF- B activation and IL-8 expression in alveolar macrophages. Second, our preliminary results showed that inhibitors of PI3K blocked activation of Rho and NF- B induced by LPS and preliminary data also demonstrated that LPS-stimulated MEKK1 activity was blocked by inhibition of RhoA GTPase. We will, therefore, confirm and extend these results by using several complementary approaches to establish the role of PI3K pathway in RhoA activity as well as define their relationship to RhoA and to the possible downstream signaling molecule (MEKK1) that may be responsible for phosphorylation of I B and IL-8 gene expression. Third, A major advance in our understanding of LPS-mediated inflammation was the discovery that the Toll-like receptor 4 gene (TLR4) encodes the LPS receptor and transduces the effect of LPS stimulation. We propose to determine whether mutations in the TLR4 can account for some of differences in LPS-stimulated signaling linked to activation of NF- B.

Grant: 1R01HL069452-01
Program Director: GANGULY, PANKAJ
Principal Investigator: HAWIGER, JACK J MD INTERNAL
MED:HEMATOLOGY
Title: Superantigen-Induced Vascular Injury and DIC
Institution: VANDERBILT UNIVERSITY NASHVILLE, TN
Project Period: 2001/09/30-2005/07/31

DESCRIPTION (provided by applicant): Superantigens are extremely potent stimulators of T lymphocytes that produce a myriad of proinflammatory cytokines known to induce vascular injury, collapse of vascular system (toxic shock syndrome), and disseminated intravascular coagulation (DIC). The mechanisms by which superantigens induce cytokine production in T cells and subsequent cytokine-evoked injury of vascular system and DIC remain to be elucidated. This highly focused and integrated research plan evolves from recent advances made by us in studying superantigen-induced vascular injury, DIC, and apoptotic organ damage. We propose a series of interrelated studies focused on the mechanism of superantigen-induced signaling in T cells subsets, Th1 and Natural Killer T cells (NKT cells). Superantigen-induced signaling mediated by NF-kappaB and other stress-responsive transcription factors will be studied in T cells and NKT cells to delineate the mechanisms responsible for their death or survival. Their interaction with endothelial cells that express superantigen-binding MHC Class II molecules will be elucidated. Cytokine-induced expression of genes encoding procoagulant proteins, tissue factor and plasminogen activator inhibitor, and their role in superantigen-induced microvascular thrombosis manifested by DIC will be delineated. Finally, cell-permeable peptides and proteins that affect superantigen-induced signaling will be designed and tested in animal models for their efficiency to ameliorate vascular injury, DIC, and apoptotic organ damage. Based on this overall workscope, this grant application is submitted in response to RFA HL-01-003 entitled "Cardiovascular, Lung, and Blood Immunobiology in Health and Disease".

Grant: 1R01HL069503-01
Program Director: MUSSON, ROBERT A.
Principal Investigator: HAJJAR, ADELINE M DVM
Title: TLR4 and Pulmonary Innate Immunity to P.aeruginosa
Institution: UNIVERSITY OF WASHINGTON SEATTLE, WA
Project Period: 2001/09/30-2005/07/31

DESCRIPTION (provided by applicant): Patients with cystic fibrosis (CF) develop persistent inflammation and chronic infections with *Pseudomonas aeruginosa* that ultimately result in their death. Toll-like receptors (TLRs) are type-I transmembrane proteins that transduce signals triggering the inflammatory and innate immune response to a variety of pathogens. TLR4, in concert with a co-receptor, MD-2, has been shown to mediate responses to lipopolysaccharide (LPS), a pro-inflammatory component of Gram-negative bacteria. This proposal will address the role that TLR4 plays in the pulmonary immune and inflammatory response to *P. aeruginosa*. The rationale for these studies derives from several novel observations made by our group. We have found that the structure of *P. aeruginosa* LPS isolated from CF patients is distinct from that of *P. aeruginosa* isolated from non-CF patients. Our preliminary in vitro data suggest that there are differences in the recognition of *P. aeruginosa* LPS by murine (mu) as compared to human (hu) TLR4. Specifically, muTLR4 mediates equivalent responses to both LPS from CF strains (CF-specific LPS) and non-CF strains (unmodified LPS) of *P. aeruginosa*, whereas huTLR4 responds much more poorly to non-CF than CF LPS. We hypothesize that CF LPS directly contributes to the chronic inflammation seen in CF and does so in part due to the unique recognition specificity of huTLR4, which recognizes and responds to CF LPS much more intensely than to unmodified LPS. The rapid and intense inflammatory response to *P. aeruginosa* in mouse lungs has limited the usefulness of mouse models for CF, possibly due to the more efficient recognition of unmodified LPS by muTLR4. We therefore propose to engineer a mouse that will mimic the decreased responsiveness of huTLR4 to unmodified LPS in order to evaluate the role that TLR4 plays in the immune response to *P. aeruginosa*.

Grant: 1R01HL069763-01
Program Director: HARABIN, ANDREA L.
Principal Investigator: RUSSO, THOMAS A MD
Title: BACTERIAL MODULATION OF LUNG INFLAMMATORY RESPONSE
Institution: STATE UNIVERSITY OF NEW YORK AT BUFFALO, NY
BUFFALO
Project Period: 2001/07/01-2005/05/31

DESCRIPTION (Unedited Applicant's Abstract): Gram-negative bacilli (GNB) are pathogens that are capable of causing severe, life-threatening pneumonia. More than 60 percent of nosocomial pneumonias are caused by GNB and associated mortality rates are often >50 percent. Over the last 10-15 years, there has been little improvement in outcome from this infection. As a result, this syndrome continues to cause significant morbidity and mortality and strongly contributes to the economic burden of our national health care system. The successful use of immune intervention in the treatment or modulation of infections has marked the beginning of a new era in the management of infectious diseases. There exists a delicate balance between an efficacious and injurious host defense response. An understanding of the host response in GNB pneumonia and how bacterial components affect this response will, in turn, lead to the development of rapid diagnostic tests that will enable the clinician to effectively utilize a variety of biologic modulators. It is also necessary to understand the relative role of bacterial components versus host factors in mediating damage to the lungs prior to therapeutic manipulations on which little is known. This information will enable us to appreciate the relative risk benefit ratio of altering the host response. Further, a more precise clarification of which host components are damaged is also needed. This knowledge may identify independent therapeutic interventions. Their global hypothesis is that surface components of GNB and/or secreted proteins differentially alter host antibacterial defenses and directly, and/or indirectly (by inflammatory mechanisms) promote lung injury. Preliminary data supports this hypothesis. These responses will have significant implications when attempting therapeutic immune interventions. The goals of this proposal are to determine the mechanisms by which the bacterial capsule and O-specific antigen modulate neutrophil recruitment into the lungs in a diametrical manner and extend our evaluations on the relative roles of bacterial factors (e.g. hemolysin) and bacterially induced host response elements in directly mediating the pathogenesis of lung injury.

Grant: 1R01HL069821-01
Program Director: HARABIN, ANDREA L.
Principal Investigator: CALLAHAN, LEIGH A MD
Title: Diaphragm Mitochondrial Alterations in Sepsis
Institution: UNIVERSITY OF ROCHESTER ROCHESTER, NY
Project Period: 2001/06/01-2005/05/31

DESCRIPTION (Applicant's abstract): Recent work suggests that mitochondria dysfunction plays a central role in sepsis, a major cause of death and morbidity in the United States. The underlying mechanisms responsible for this mitochondria dysfunction are not known. The goal of the present proposal is to test the hypothesis that increased free radical generation in sepsis produces specific biochemical, structural and genetic changes that result in marked physiologic alterations in mitochondrial function. We postulate: (a) mitochondria dysfunction in sepsis results from physiologic derangements of Krebs cycle enzymes, Complex I-IV electron transport chain components, and sarcomeric creatine kinase, (b) these physiologic changes are due, in turn, to alterations in the content and composition of mitochondrial proteins, and (c) protein changes are due, in part, to free radical-mediated decrements in mitochondrial gene transcription, expression, and translation. These hypotheses will be tested in three groups of experiments, using a model of endotoxin-induced sepsis. The purpose of Objective 1 is to fully characterize the specific physiologic derangements in the mitochondria in sepsis; we will examine Krebs cycle enzyme activities, evaluate specific performance of complexes within the electron transport chain, assess sarcomeric mitochondrial creatine kinase activity, and perform a metabolic control analysis. Objective II will identify changes in the content and composition of mitochondrial protein constituents (i.e. electron transport chain protein subunits, Krebs cycle enzymes, creatine kinase) and compare the time course of these alterations with the development of physiologic abnormalities determined in Objective I. Objective III will evaluate transcription, expression, and translation of mitochondria and nuclear genes encoding for mitochondrial proteins found to be depleted in Objective II. In all studies, we will determine the role of free radical modulation of these sepsis-induced changes. Our preliminary data provide the first evidence of substantial sepsis-associated oxidative modification and depletion of mitochondria protein subunits in Complexes I, III and IV, significant alterations in NADH generation via Krebs cycle enzymes, major decreases in mitochondria creatine kinase activity, and key free radical-mediated changes in gene expression of mitochondrial proteins in sepsis. These data suggest that the proposed experiments should provide important information regarding the pathogenesis of mitochondrial dysfunction in sepsis.

Grant: 1R03HL067427-01
Program Director: NOEL, PATRICIA
Principal Investigator: OWNBY, DENNIS R MD MEDICINE
Title: Antibiotics in Infancy-Risk Factor for Childhood Asthma
Institution: MEDICAL COLLEGE OF GEORGIA (MCG) AUGUSTA, GA
Project Period: 2001/04/01-2003/03/31

DESCRIPTION (Provided by Applicant) Morbidity and mortality from childhood asthma have been increasing in all developed countries over the past three decades, including in the United States. Numerous theories have been advanced to explain this asthma epidemic, but no single theory has held up to careful scrutiny. Recent international studies have suggested a relatively strong causal relationship between increased risk of childhood asthma and exposure to antibiotics during childhood, especially during the first year of life. The increased asthma risk was seen whether antibiotics were used to treat respiratory or non-respiratory infections. While these previous studies are suggestive, there are significant methodologic concerns about each study. A major concern with most of the studies is their reliance on retrospective recall of antibiotic exposure data from parents years after the exposure. We have data from a prospective, NIH-funded study of the relationship between early environmental exposures and the development of asthma in a birth cohort of children followed to an average 6.7 years of age. At 6.7 years, 482 (58%) of the original 833 children were clinically examined as part of this Childhood Asthma Study (CAS). In addition to clinical histories, the 6- to 7- year clinical examination included skin tests, IgE antibody tests, pulmonary function tests and methacholine challenge. At entry all of the CAS children were within the Health Alliance Plan (HAP) HMO. The current proposal is based on combining the CAS data set with pharmacy data extracted from the HAP data archives. This will allow us to examine possible relationships between antibiotic use, as determined by prescriptions filled, and asthma at 6 to 7 years of age. While not strictly a prospective study, these methods will avoid many of the potential sources of bias found in previous studies. We will also be able to evaluate any relationships between antibiotic exposure and asthma for confounding by other risk factors such as bedroom allergen levels, pet ownership, cigarette smoke exposure, and parental history of asthma or allergy. The proposed study is entirely separate from the goals of the original grant which did not consider antibiotic use as a potential risk factor for asthma or allergy. This new analysis will allow a much more rigorous examination of the possible relationship between early antibiotic use and asthma in a population of American children.

Grant: 2R01MH043778-10A1
Program Director: JOSEPH, JEYMOHAN
Principal Investigator: NANCE, DWIGHT M PHD
Title: Neural Control of Macrophages by Endotoxin and HIV-1
Institution: UNIVERSITY OF MANITOBA WINNIPEG, MB
Project Period: 2001/06/20-2002/01/31

Our objective is to identify the neural substrates and mechanisms that mediate the neural control of the immune system. The proposed neuroimmune model states that the immune system signals the brain and the CNS subsequently modifies peripheral immune function. We showed that an immune challenge activates central autonomic regulatory regions, increases sympathetic output to the spleen, and modifies splenic immune function via the sympathetic splenic nerve. Similarly, stress activates the neural-immune regulatory circuit and also suppresses splenic macrophage function via the splenic nerve. We propose that immune signals and stress modify CNS activity via a specific signal transduction cascade and activation of this cascade alters autonomic and endocrine regulatory circuits which then signal the immune system via definable pathways and chemical mediators. HIV-1 may disrupt this neural-immune regulatory circuit and we hypothesize it produces autonomic dysregulation and disarms the neural regulation of the immune system. Our aim is to identify the neuroanatomical and neurochemical mechanisms mediating the effects of endotoxin, stress, and the HIV-1 viral coat protein gp120 on splenic macrophage function. Central induction of c-fos protein and multiunit electrical activity in the brain will index activation of the central circuit by immune stimuli and nerve recordings will measure sympathetic output to the spleen. In vivo splenic macrophage cytokine production following an endotoxin challenge will measure immune function and corticosterone levels will index endocrine activation. Intracranial injections of gp120, prostaglandins, neuropeptide and nitric oxide agonist and antagonist or stress will be combined with an endotoxin challenge to establish the organization of the signalling cascade and to determine if it is a target for gp120. Finally, simultaneous brain and splenic nerve recordings will monitor the activation and output of the regulatory system following systemic and central injections and provide a direct link between the central activation of the neural-immune axis and alterations in splenic macrophage function. These experiments will characterize the functional pathways from the brain to the spleen and determine the effects of gp120 on this regulatory system.

Grant: 1R01MH060706-01A2
Program Director: WINSKY, LOIS M.
Principal Investigator: KUSNECOV, ALEXANDER W PHD
Title: Neural and Behavioral Impact of T Cell Activation
Institution: RUTGERS THE ST UNIV OF NJ NEW PISCATAWAY, NJ
BRUNSWICK
Project Period: 2001/05/01-2004/04/30

DESCRIPTION: Considerable neuropsychiatric problems result from cytokine immunotherapy (eg., interleukin-2: IL-2), which may reflect differences in the way the brain responds to exogenous cytokines as opposed to endogenous cytokines elicited by antigens as part of a dynamic cytokine network. Therefore, the current project aims to understand how the brain reacts to endogenous cytokine production induced by Staphylococcal enterotoxin B (SEB). This model involves activation of a natural repertoire of immunological events that serves to regulate not only immune responses, but how the organism should perform adaptive behavioral adjustments (eg., during sickness). Administration of SEB in vivo stimulates T cells to produce high levels of IL-2 and tumor necrosis factor-alpha (TNF-a), and this has been associated with increased transcription of central corticotropin releasing hormone (CRH) in the central nucleus of the amygdala, (ceA) and paraventricular nucleus (PVN) of the hypothalamus, which was associated with increased neophobic reactivity. Immunoneutralization of systemic IL-2 significantly attenuated the impact of SEB challenge on neuronal activation in the central nucleus of the amygdala and adjacent limbic regions of the brain. Therefore, in Specific Aim 1 we will test whether SEB challenge promotes enhanced reactivity to anxiety-provoking stimuli. These will include ethological tests of anxiety and/or fear (viz., open field and elevated plus maze) under familiar and novel contextual conditions. In addition, SEB challenged animals will be tested for learned fear responses, such as fear potentiated startle reactivity, a behavior that is strongly linked to the amygdala. Specific Aim 2 will determine the role of IL-2 and TNF-a in the behavioral effects of SEB. Studies will involve anti-immune cytokine monoclonal antibody treatment, and in the case of studying IL-2, SEB challenge of mice possessing IL-2 gene mutations. Specific Aim 2 will also test whether the SEB-induced CRH mRNA increases in the amygdala and other brain regions are mediated by the aforementioned cytokine manipulations. Finally, in Specific Aim 3 the role of CRH in promoting the behavioral effects of SEB will be tested by site-specific administration of CRH receptor antagonists into the ceA and locus coeruleus, regions known to mediate some of the anxiogenic effects of CRH. These studies will confirm if immunological activation modifies CNS reactivity to psychological stressors, which in turn may promote efforts to understand how T cell derived cytokines (such as IL-2 and TNF-a) support and/or influence behavioral adaptation to stress. In view of the increasing emphasis on the role of the immune system in affecting motivational systems typically altered in clinical depression, this research will contribute to understanding the aetiology and strategies for treatment of affective illness.

Grant: 1R01MH061940-01A1

Program Director: DELCARMEN-WIGGINS, REBECCA

Principal Investigator: LECKMAN, JAMES F MD INTERNAL
MED:INTERNAL MEDICINE
UNSPEC

Title: A Prospective Longitudinal Study of PANDAS

Institution: YALE UNIVERSITY NEW HAVEN, CT

Project Period: 2001/09/27-2006/08/31

DESCRIPTION (provided by applicant): Tic disorders, obsessive-compulsive disorder (OCD), and related conditions are prevalent disorders affecting as many as 0.3-3 percent of the pediatric population. They are chronic, relapsing disorders that can be 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 etiologies of these disorders are unknown. It has been hypothesized that susceptible individuals develop symptoms of these disorders as a result of post-infectious autoimmune processes. Infections with group A beta hemolytic streptococci (GABHS) are thought to initiate these processes. Swedo, Leonard and colleagues (1998) have proposed that this subgroup of tic disorder patients, identified by the acronym PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections) follows a unique clinical course. Although this hypothesis is strengthened by: (1) the presence of potentially crossreactive antineuronal antibodies in the sera of a minority of patients with tic disorders and/or OCD; (2) enlarged basal ganglia in PANDAS cases; and (3) the finding that plasma exchange and intravenous immunoglobulin treatment are effective in reducing symptoms in PANDAS cases, this conceptualization remains controversial. The aim of this revised study is to validate the diagnostic concept of PANDAS by performing a prospective longitudinal study. Further cross-sectional studies cannot resolve this issue. The use of a prospective longitudinal design should permit a close examination of the timing of symptom relapses relative to GABHS exposure. Other specific aims focus on longitudinal fluctuations of psychosocial stress, anti-neuronal antibody levels and basal ganglia volumes in an effort to understand the pathobiology of PANDAS. If specific factors are associated with acute relapse, then the nature of these factors should provide insight into the immunologic mechanisms involved. This knowledge may provide a basis for the rational design of therapeutic and preventative interventions. More generally, the knowledge gained should advance our models of disease pathogenesis and clarify the interaction between psychosocial stress and psychoneuroimmunological mechanisms. These insights may be relevant to our understanding of other diseases including AIDS.

Grant: 1R01NS040730-01
Program Director: KERZA-KWIATECKI, A P
Principal Investigator: KIELIAN, TAMMY L PHD MICROBIOLOGY, OTTUMWA, IL
Title: THE PATHOGENESIS OF BRAIN ABSCESS
Institution: DARTMOUTH COLLEGE HANOVER, NH
Project Period: 2001/01/05-2001/06/30

DESCRIPTION: (adapted from applicant's abstract) The focus of this proposal is to understand the immune response to pyogenic bacteria, such as *S. aureus*, in the pathogenesis of experimental brain abscess. This study may lead to identification of host and bacterial factors that play a key role in brain abscess development and pathology. First, they will evaluate, using *S. aureus* (one of the main etiological agents of brain abscess in humans,) the host immune responses in the central nervous system (CNS) parenchyma. They will use a rodent experimental model, and examine cytokines and chemokines elicited in response to bacterial challenge, the cellular origin(s) of these mediators within the CNS, and the response of primary astrocytes and microglia. Next, they will evaluate the relation of bacterial virulence factors in abscess pathogenesis. They will evaluate the role of virulence factors expressed in the CNS and what effect they have on the host immune response to infection.

Grant: 1R01NS042194-01
Program Director: UTZ, URSULA
Principal Investigator: ASCHERIO, ALBERTO MD
Title: Pre-diagnostic markers of infection and risk of MS
Institution: HARVARD UNIVERSITY (SCH OF PUBLIC BOSTON, MA
HLTH)
Project Period: 2001/08/15-2004/07/31

We propose to conduct a prospective investigation to assess whether infection with the Epstein-Barr Virus or Chlamydia pneumoniae increases the risk of multiple sclerosis (MS). For this purpose we have identified two large populations of individuals whose blood samples were collected and stored several years ago and are available for analyses, and we are in the process of documenting cases of MS that occurred in these populations after the date of blood collection. One population comprises over 3 million US Army personnel whose blood samples are stored in the Department of Defense (DOD) Serum Repository; the other comprises 125,000 participants in the Kaiser Permanente Health Plan (KPHP), whose blood samples were collected over 20 years ago. The identification and diagnostic confirmation of the cases of MS occurring in these populations has already been funded in part by a pilot grant from the National Multiple Sclerosis Foundation. Based on our preliminary work, we estimate that we will be able to document 216 cases of MS with onset after the date of collection of the stored serum samples. Main hypotheses to be addressed are that risk of MS is increased among individuals infected with EBV or C pneumoniae, as determined by the presence of specific serum antibodies, and that elevated antibody titers against EBV or C pneumoniae antigens predate the onset of MS.

Grant: 1R01NS042240-01
Program Director: FINKELSTEIN, ROBERT
Principal Investigator: KURLAN, ROGER M
Title: PANDAS and Strep Infection: Are they Linked?
Institution: UNIVERSITY OF ROCHESTER ROCHESTER, NY
Project Period: 2001/09/25-2005/07/31

Sydenham's chorea is the only accepted immune-mediated central nervous system manifestation of group A beta hemolytic streptococcal (GABHS) infection. Several lines of evidence now suggest that there may be a spectrum of post-GABHS immune-mediated neurobehavioral sequelae termed "Pediatric Autoimmune Neuropsychiatric Disorders after Streptococcal Infection (PANDAS)". Tics, including Tourette's syndrome (TS), and obsessive-compulsive disorder (OCD) have been reported as the characteristic features of PANDAS. Proposed is a multicenter prospective case control cohort study involving 40 cases of PANDAS and 40 matched controls with TS and/or OCD but without evidence of PANDAS. All subjects will undergo intensive clinical and laboratory prospective observation for 24 months to determine whether antecedent GABHS infection is: 1) temporally associated with exacerbations of PANDAS, 2) specifically associated with exacerbations of tics and OCD, and 3) a specific trigger for exacerbations of PANDAS. All determinations of case/control status, GABHS infection and clinical exacerbation will be determined independently by blinded review in order to limit selection and clinical biases. Establishing a post-infectious etiology for PANDAS would dramatically change our understanding of the causes of TS and OCD, alter our therapeutic approach and may have a critical public health implication of preventing potentially fatal rheumatic cardiac sequelae in affected children. Disproving the PANDAS hypothesis would prevent the use of expensive and potentially dangerous therapies (e.g., plasma exchange, immune globulin, antibiotics) that have been proposed for PANDAS patients.

Grant: 1R01NS044000-01
Program Director: KERZA-KWIATECKI, A P
Principal Investigator: BASTIAN, FRANK O MD
Title: Spiroplasma 16S rDNA in TSE Brain Tissues
Institution: TULANE UNIVERSITY OF LOUISIANA NEW ORLEANS, LA
Project Period: 2001/09/30-2004/07/31

DESCRIPTION (Adapted from applicant's abstract): The pathogenesis of the transmissible spongiform encephalopathies (TSE), which include Creutzfeldt Jakob disease (CJD) in humans and scrapie in sheep, remains an enigma. In this application we present evidence for the association of Spiroplasma sp., a wall-less prokaryote, with TSE. We have shown PCR amplification of Spiroplasma 16S rDNA in TSE-infected brain tissues (19) and not in control brains (0 of 50). Direct sequencing of the amplified PCR products has confirmed the presence of Spiroplasma-like DNA in all 19 of the TSE brains. Our evidence is not necessarily in conflict with involvement of a PrPres, a protease resistant host derived protein referred to as the prion, in the pathogenesis of TSE, since there is evidence that another factor is involved, which we propose to be a bacterium. Our strategy will be to optimize our probe by characterizing the entire Spiroplasma ribosomal gene in TSE and, then, screen a statistically significant number of TSE cases. In Aim 1, we will utilize a combination of universal Mollicute 16S rDNA oligonucleotide primers along with Spiroplasma-specific 16S rDNA primers to identify by PCR the near complete Spiroplasma 16S RNA gene in TSE. Direct sequencing of the PCR products will ascertain the unknown adjacent portions of the Spiroplasma-like ribosomal gene involved in TSE, from which we will design new oligonucleotide primer set/s that will detect all Spiroplasma strains, including those associated with TSE. In Aim 2 we will screen, with the new probe/s, a sizable collection of TSE cases, including 25 available CJD human cases and 105 scrapie-infected and normal sheep brains. An additional 100 brain samples, both TSE-infected and normals, are to be sent by collaborators as randomly coded samples. The PCR amplified products in all instances will be sequenced to determine the precise nature of the Spiroplasma sp. involved. Although the role of Spiroplasma in TSE cannot be determined from these experiments, the presence of this microbe in all cases of TSE and not in controls would provide the basis for developing a test for TSE

Grant: 1R21NS041660-01
Program Director: LEBLANC, GABRIELLE G
Principal Investigator: FISHMAN, PAUL S PHD
Title: Motor Neuron Protection through Glutamate Degradation
Institution: UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD
SCHOOL
Project Period: 2001/08/01-2003/07/31

DESCRIPTION (provided by applicant): Glutamate-provoked excitotoxicity may contribute to the pathogenesis of both acute and degenerative neurological disease. Although glutamate re-uptake is the normal manner through which the action of the neurotransmitter is terminated, glutamate can also be eliminated through enzymatic degradation. We have recently created a functionally active hybrid protein that links a glutamate-degrading enzyme (glutamate-pyruvate-transaminase, GPT-also known as ALT) with the non-toxic neuronal binding domain of tetanus toxin (tetanus toxin fragment C or TTC). The rationale for producing this molecule is to deliver an enhanced capacity to enzymatically-degrade glutamate to synaptic regions surrounding motor neurons, and thereby protect them from toxic levels of glutamate. Our previous studies have demonstrated: 1) TTC can dramatically enhance (100 to 1,000 fold) the delivery of active enzyme to neurons in vitro and motor neurons in vivo. 2) TTC-linked enzymes are targeted to synaptic regions surrounding neurons in vitro and motor neurons in vivo. 3) GPT is the most effective enzyme to rapidly (within minutes) reduce neurotoxic levels of glutamate. 4) GPT can protect neurons in vitro from both direct exposure to toxic levels of glutamate and the toxic effect of inhibition of glutamate re-uptake. We propose to assess TTC-GPT in models of chronic glutamate excitotoxicity and motor neuron degeneration. Initially, we will quantitate the uptake and persistence of active enzymes to explant cultures of neo-natal rat spinal cord with both native GPT and TTC-GPT. We will then assess the capacity of TTC-GPT to prevent progressive motor neuronal death in these spinal cord cultures exposed to inhibitors of glutamate re-uptake, or after reduction of synthesis of high-affinity glutamate transporters using antisense oligonucleotides. We will assess the capacity of TTC-GPT to deliver active enzymes to motor neurons from an intramuscular injection, via retrograde axonal transport, in both normal mice and in a murine model of familial ALS. This proposal will determine the potential of not only glutamate degradation as an anti-excitotoxic strategy, but of TTC as a vector to deliver therapeutic proteins with synaptic sites of action. If successful, TTC-GPT can be assessed in animal models of motor neuron disease, such as the FALS mouse, in the future. These studies will advance understanding of the potential of a novel means of glutamate elimination with therapeutic implications of diseases for not only ALS, but also other neurologic diseases such as stroke, Alzheimer's Disease and Parkinson's Disease.

Grant: 1R01RR014298-01A2
Program Director: GRIEDER, FRANZISKA B.
Principal Investigator: SOLNICK, JAY V MD
Title: Transmission of H. pylori infection in the rhesus monkey
Institution: UNIVERSITY OF CALIFORNIA DAVIS DAVIS, CA
Project Period: 2001/09/12-2006/08/31

DESCRIPTION (provided by the applicant): Infection with *Helicobacter pylori* causes a histological gastritis that in some individuals is associated with the development of peptic ulcer disease or gastric malignancy. Although *H. pylori* may be the most common human bacterial infection, the mechanism by which it is transmitted remains unknown. Person to person transmission probably accounts for most infections. Yet one of the great paradoxes in the epidemiology of *H. pylori* is that when one examines the gastric lining, the bacterium is ubiquitous, but when fecal or oral secretions are studied it is often difficult to find. This may reflect the difficulty of studying in humans the role of acuity of infection, age of the host, and the possible effects of vomiting, diarrhea, and the CagA pathogenicity island on transmission. Rhesus monkeys are naturally infected with *H. pylori* that is very similar to strains that infect humans, and this animal model provides a unique opportunity to study experimentally the transmission of *H. pylori* in a naturally infected host. We hypothesize that acuity of infection, the presence of vomiting and diarrhea, and the CagA pathogenicity island are critical variables in transmission of *H. pylori*. Furthermore, we propose that there may be a cooperativity between transmission of *H. pylori* and transmission of bacterial enteric diseases. Diarrheal and vomiting diseases may increase *H. pylori* transmission by increasing the shedding *H. pylori* in feces and vomitus, and in turn, *H. pylori* infection may cause increased gastric pH and thereby promote infection with enteric bacteria by reducing the gastric bactericidal barrier. We propose to address four specific aims in this proposal: 1) Determine how *H. pylori* is shed into the environment during acute and chronic infection; 2) Examine experimentally the effects of vomiting, diarrhea and the CagA pathogenicity island on the natural transmission of *H. pylori*; 3) Determine the effects of *H. pylori* infection on the acquisition of *Campylobacter jejuni*; and 4) Determine the effects of the CagA pathogenicity island on colonization and shedding.

Grant: 1R21RR015293-01A1
Program Director: GRIEDER, FRANZISKA B.
Principal Investigator: SOLNICK, JAY V MD
Title: Transcription of H. pylori Genes in the Rhesus Monkey
Institution: UNIVERSITY OF CALIFORNIA DAVIS DAVIS, CA
Project Period: 2001/06/20-2003/05/31

DESCRIPTION (provided by the applicant): *Helicobacter pylori* is an extremely common infection that causes gastritis and is clearly implicated in the development of peptic ulcer disease and gastric cancer. A full understanding of *H. pylori* pathogenesis requires an analysis of bacterial gene expression in vivo in a relevant animal model. The availability of the complete *H. pylori* genome sequence, together with novel technologies for measurement of gene expression, makes it possible to address this problem in the rhesus monkey model that we have recently developed at the California Regional Primate Research Center (CRPRC). We hypothesize that *H. pylori* genes important in virulence will be induced by contact with the host gastric epithelium. Furthermore, we propose that bacterial gene expression in the host will be modulated by the presence of the *cagA* pathogenicity island and by *luxS*, a gene implicated in quorum sensing. Specific pathogen free rhesus monkeys will be inoculated with wild type *H. pylori* or with isogenic mutants carrying deletions in the *cagA* pathogenicity island or in *luxS*. In vivo transcription in targeted genes will be measured by quantitative RT-PCR during a 6-month period of infection. The results in animals infected with wild type and mutant strains will be compared over time. Furthermore, we will compare changes in gene transcription between cells grown in vitro and those isolated in vivo. An understanding of the dynamic interaction between host and pathogen may point the way toward critical proteins that may be useful as targets for therapy or vaccine development.

Grant: 1R01TW005605-01
Program Director: NUGENT, RACHEL
Principal Investigator: SIMON, JONATHON L MPH
Title: The impact of Morbidity on Labor Productivity in Wester*
Institution: BOSTON UNIVERSITY MEDICAL CAMPUS BOSTON, MA
Project Period: 2001/05/19-2003/04/30

DESCRIPTION: Recent macroeconomic modeling suggests that health has a substantial impact on the growth of per capita GDP (Gross Domestic Product) and is, thus, a critical input to economic development. The macroeconomic mechanisms by which a healthier population contributes to a country's economic growth, however, are poorly understood. One important pathway about which we know little is the impact of adult morbidity on labor productivity. The specific aim of the proposed study is to assess the impact of major acute and chronic adult morbidities on agricultural labor productivity in rural sub-Saharan Africa. It will answer two key research questions: 1) What is the impact of malaria, HIV/AIDS, and tuberculosis on individual labor productivity? and 2) To what extent does adult morbidity hinder economic development through its impact on wages and profits? The study will also create opportunities to strengthen local capacity to conduct interdisciplinary research on the relationship between health and development. The study will use retrospective and prospective data from a group of large agricultural estates in western Kenya, including individual daily output (weight of crop harvested per day) records and detailed morbidity data. By linking these two data sets, the study will be able to observe directly both productivity and health, overcoming a major limitation of most previous research. The data set is expected to include approximately 870 cases of malaria, 420 cases of HIV, and 430 cases of tuberculosis; each case will have an average of 270 daily productivity observations per year. These data will allow the study to determine the effect of morbidity on three aspects of productivity: reduced performance on the job, absenteeism, and the loss of workforce experience when a sick worker leaves the workforce prematurely. Productivity losses will be assessed from the date of infection to full recovery or departure from the workforce. The study will also measure the effects of concurrent infections with HIV and malaria or HIV and TB and will describe the relationship between biomedical indicators of disease (CD4 [a member of the immunoglobulin superfamily] counts, malaria parasite densities, and lib levels) and labor productivity. The results of the productivity analysis will be used to estimate the impact of adult morbidity on wage income and firm profits, two of the most important components of gross domestic product. The study will also estimate the extent to which labor substitution offsets the impacts on wages and profits. The study is distinguished by the opportunity to link a large amount of individual productivity data to a medical records system with good diagnostic capabilities, which will allow it to generate empirically robust estimates of the impact of common adult morbidities at the macroeconomic level. The study will help explain whether and how the impacts of adult morbidity on wages and profits can generate the macroeconomic effects reported in recent cross-country analyses. It will thus help to fill a critical gap in our knowledge about the relationship between health and economic development.

Grant: 1R03TW001319-01A1
Program Director: SINA, BARBARA J
Principal Investigator: COX, MICHAEL M
Title: Structure/function of RecA protein from *P. aeruginosa*
Institution: UNIVERSITY OF WISCONSIN MADISON MADISON, WI
Project Period: 2001/05/01-2004/04/30

The RecA protein of *E. coli* promotes a DNA strand exchange reaction in vitro that provides a convenient molecular model for the central steps of recombinational DNA repair and homologous genetic recombination. The long-range goal of the research in RO1 GM32335 (Cox) is a detailed understanding of RecA-mediated DNA strand exchange. The hypothesis that recombinational DNA repair is the primary function of RecA protein in vivo provides an intellectual framework. One of the specific aims of GM32335-17 (recently funded) is to carry out a comparative analysis of bacterial RecA proteins and RecA homologues. *Pseudomonas aeruginosa*, an important human pathogen, is the source of one of the RecA proteins to be investigated. In the present proposal, a collaboration is proposed that should greatly extend our proposed analysis of the *Pseudomonas aeruginosa* RecA protein, and improve the prospects for mechanistic and structural insights. Together with the laboratory of Dr. Vladislava Lanzov in St. Petersburg, Russia, we will first explore the biochemistry of the *P. aeruginosa* RecA protein, and establish enzymatic differences between it and the *E. coli* RecA. Using an extensive set of active chimeric proteins constructed from fusions of the two RecA proteins, we will try to pinpoint regions of the protein responsible for the differences. The work should help test key features of current models for RecA-mediated DNA strand exchange, and may also help identify RecA variants with enhanced DNA binding and strand exchange functions. Such proteins may eventually prove useful in efforts to use RecA in gene therapy protocols and to generate crystals of RecA-DNA complexes for structural analysis.

Grant: 1R03TW001354-01A1
Program Director: KATZ, FLORA
Principal Investigator: WIPF, PETER
Title: Synthesis and Biology of Bengazole Analogs
Institution: UNIVERSITY OF PITTSBURGH AT PITTSBURGH, PA
PITTSBURGH
Project Period: 2001/05/01-2004/04/30

This is an application for a Fogarty International Research Collaboration Award (FIRCA) between the research groups of Dr. Gloria Serra (Assistant Professor of Medicinal Chemistry, Universidad de la Republica, Montevideo, Uruguay) and Dr. Peter Wipf (Professor of Chemistry, University of Pittsburgh, Pittsburgh, PA, USA). Our specific aims in this collaborative project are: - Synthesis of bis-oxazoles, bis-oxazolines, bis-thiazoles and bis-thiazolines analogs of the anthelmintic marine natural product bengazole. This is the primary goal of this application. - Evaluation of biological activities, e.g. i) In vitro anthelmintic activity against *Nippostrongylus brasiliensis* by Prof. Laura Domínguez from the Pharmacology Department of the Faculty of Chemistry (Uruguay); ii) cytotoxic activity in cancer cell lines by Dr. Adriana Baz at the Immunology Department of the Faculty of Chemistry (Uruguay); iii) if the results of the in vitro anthelmintic activity assays are significant, the Pharmacology Department will perform an in vivo evaluation. - SAR analysis of compounds of type 1: i) esterification with fatty acids of some of the OH groups on the lateral chain; ii) preparation of O/S heterocycle analogs for structures of type 1; iii) preparation of analogs differing in the level of polyhydroxylation at the lateral chain R. Chemotherapy has been a long-standing effective instrument for battling parasitic infections in both human and veterinary medicine. Although there have been major advances in the clinical treatment of anthelmintic diseases, there are still no ideal broad-spectrum anthelmintics. Moreover, the field has suffered major setbacks, since resistance of nematodes to the anthelmintic drugs of general use such as benzimidazoles levamisole and praziquantel has emerged. The proposed project will provide a wide range of bengazole analogs for testing of their anthelmintic properties which represents a crucial step in the development of new antiparasitic pharmaceuticals.