

**FY04**  
**NIH Extramural Support**  
**in Bacteriology Research**

Includes Research Project Grants (RPGs)  
Excludes clinical trials

**Grant:** 2R01AA011760-06A2  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** MASON, CAROL M MD  
**Title:** Alcohol and Reactivation TB  
**Institution:** LOUISIANA STATE UNIV HSC NEW ORLEANS NEW ORLEANS, LA  
**Project Period:** 1997/09/25-2008/12/31

**DESCRIPTION** (provided by applicant): Tuberculosis, due to infection with *Mycobacterium tuberculosis*, is the most prevalent infection globally, with up to 40% of the world's population (1.8 billion people) infected. The vast majority of these infections are latent (chronically persistent in the setting of established antimycobacterial immunity), a stage which is critically dependent upon CD4<sup>+</sup> lymphocytes. Several recognized factors result in the breakdown of established immunity, leading to reactivation of the infection and overt disease, including concurrent HIV infection or adverse socioeconomic conditions. The latter includes alcohol abuse. Alcohol adversely impacts the immune system, including lymphocytes. In murine models, vaccination with *M. bovis* Bacille Calmette-Guerin (BCG) followed by lung infection with *M. tuberculosis* models latent infection (by resulting in chronic persistent infection). Based on our work in the prior funding period, we hypothesize that alcohol consumption impairs *M. bovis* BCG-induced protection against pulmonary infection with *M. tuberculosis* in mice by disrupting effector CD4<sup>+</sup> Th1 lymphocyte responses that are necessary to control the proliferation of *M. tuberculosis*. This will be tested in a murine model of chronic alcohol consumption and pulmonary infection with virulent *M. tuberculosis* after immunization with BCG. The Specific Aims are: Specific Aim 1. To test the hypothesis that alcohol consumption by mice will disrupt *M. bovis* BCG-induced CD4<sup>+</sup> Th1 effector lymphocyte (vs. cognate) immunity against pulmonary infection with *M. tuberculosis*. Specific Aim 2. To test the hypothesis that alcohol-induced disruption of *M. bovis* BCG induced immunity against pulmonary infection with *M. tuberculosis* is due to impaired expansion, recruitment, and/or function of CD4<sup>+</sup> Th1 lymphocytes. Specific Aim 3. To test the concept that alcohol-induced defects in BCG-induced protection against pulmonary challenge with *M. tuberculosis* can be reconstituted via immunomodulation (i.e., cytokine gene therapy). The goal of the proposed project is to elucidate the specific effects of alcohol consumption on the breakdown of established antimycobacterial immunity, and to develop strategies to prevent these events

<b>Grant:</b>	1R21AA014796-01	
<b>Program Director:</b>	BRYANT, KENDALL	
<b>Principal Investigator:</b>	ANTONY, VEENA B	MBBS
		PHYSIOLOGY:PHYSIOLOGY-UNSPEC
<b>Title:</b>	HIV, Alcohol and TB Pleurisy	
<b>Institution:</b>	UNIVERSITY OF FLORIDA	GAINESVILLE, FL
<b>Project Period:</b>	2004/09/06-2007/08/30	

DESCRIPTION (provided by applicant): Alcohol abuse and the Human immunodeficiency virus (HIV) are both known to compromise normal immune responses to infections. Pleuropulmonary tuberculosis is a major health problem, particularly in developing countries and is commonly found in patients who have AIDS or those who abuse alcohol. Alcohol-induced tissue injury and cellular stress further compromise normal protective responses. Heme oxygenase (HO) is a microsomal stress-induced enzyme that is cytoprotective through the release of its metabolic products, ferritin, bilirubin and carbon monoxide. In our preliminary studies, we demonstrate that in pleural tuberculosis, the inducible form of HO (HO-1) is inhibited by alcohol. Based on background information and our preliminary data, we hypothesize that: Chronic alcohol abuse causes inhibition of heme oxygenase -1 (HO-1) enzyme expression that leads to increased cellular stress, poor granuloma formation and dissemination of mycobacteria in patients with pleuropulmonary tuberculosis. We will evaluate our hypothesis in the following specific aims: Specific Aim 1: To evaluate the prevalence of alcohol abuse and HIV status in patients with pleural tuberculosis in Chandigarh, India and to evaluate if these patients have decreased HO-1 and its metabolic end products in pleural inflammatory cells and fluids. Specific Aim 2: To evaluate if chronic alcohol administration to mice with pleural TB causes decreased HO-1 expression, in inflammatory and resident cells, decreased granuloma formation and increased dissemination of mycobacteria. Specific Aim 3. To evaluate if alcohol alters mycobacteria-induced pleural mesothelial permeability and barrier function in vitro through HO-1- mediated mechanisms. Addressing these specific aims will allow us to collect important information in a resource poor society, and better understand the role of alcohol in increasing susceptibility to pleuropulmonary tuberculosis.

**Grant:** 1R01AG021097-01A2  
**Program Director:** QUILL, HELEN R.  
**Principal Investigator:** TURNER, JOANNE PHD  
**Title:** CD8 T cells and immunity to tuberculosis in old mice  
**Institution:** OHIO STATE UNIVERSITY COLUMBUS, OH  
**Project Period:** 2004/09/30-2009/07/31

**DESCRIPTION** (provided by applicant): The elderly are more susceptible to many infectious diseases, and yet vaccinating this population is less effective when vaccines that are designed for young individuals are used. To design a vaccine or post-exposure therapy that can protect the elderly against infectious disease it is first necessary to understand how the aging immune response differs from younger individuals when it encounters a pathogen. Using the aging mouse model of tuberculosis we have found that old mice express a transient early resistance to infection that correlates with the presence of CD8 T cells within the lungs. This identifies a previously unrecognized novel immune mechanism in old mice that is clearly absent from the lungs of young mice. The CD8 T cell may therefore be a potential target population for the design of vaccines or novel post-exposure therapies for the elderly. Using the low dose aerosol infection model of tuberculosis we will characterize this CD8 T cell population further by determining when CD8 T cells become more active within the lungs of old mice and the mechanism by which CD8 T cells mediate early resistance. Studies will be carried out in a new BSL-3 facility at Colorado State University and will use old wild type, gene-disrupted, or transgenic mice from our existing in-house aging mouse colonies. The technical approaches will use a combination of flow cytometry, immuno-histochemical staining, and real-time PCR, to address the proposed aims.

**Grant:** 1P01AI055621-01A1

**Program Director:** SCHAEFER, MICHAEL R.

**Principal Investigator:** BENACH, JORGE PHD  
MICROBIOLOGY:BACTERIOLOG  
Y

**Title:** Agents of Bioterrorism: Pathogenesis and host defense

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

**Project Period:** 2004/06/15-2009/05/31

DESCRIPTION (provided by applicant): There is much concern about the possibility of renewed use of biological agents in intentional acts of bioterrorism, and the necessity to protect the public's health if renewed attempts were to become a reality. To meet this challenge, new avenues for basic research on the pathogenesis of and host defense to Category A agents of Bioterrorism have become available. In this Program Project Grant application in response to initiatives on Biodefense and Emerging Infectious Disease Research, a group of scientists with previous experience with *Francisella tularensis*, hantaviruses, and *Yersinia pestis* will collaborate in a comprehensive project that will study: 1) the mechanisms of survival and replication of *Y. pestis* in macrophages; Project 1, James Bliska. 2) the novel mechanisms of *F. tularensis* interaction with cells of the inflammatory and innate immune systems; Project 2, Martha Furie. 3) the biochemical role of the prokaryotic SmpB-SsrA quality control system of *Y. pestis* and *F. tularensis* with particular emphasis on antibacterial therapy and its utility in anti-infective drug discovery; Project 3, Wali Karzai. 4) the hantavirus proteins that regulate cellular IFN responses and define mechanisms of signaling pathway regulation by pathogenic hantaviruses; Project 4, Erich Mackow. 5) the virulence factors of *Y. pestis* and *F. tularensis* and to elucidate the mechanisms of virulence factor assembly and secretion by these pathogens; Project 5, David Thanassi. The Research Projects will be supported by three Cores. Core A will deal with all administrative, development, and laboratory security matters and it will be headed by the PI, Jorge Benach. Core B is the Microarray and Bioinformatics Core headed by Bruce Futcher will produce spotted DNA microarrays for the genomes of *F. pestis* and *F. tularensis*, and make custom human microarrays. Core B will provide advice and assistance with all microarray experiments; and with all data interpretation with state of the art Bioinformatics. Core C will produce monoclonal antibodies for all of the Projects. A multidisciplinary approach including aerosol infections of mice in a BSL3Ag setting is expected to be a powerful tool for the basic research needs of these agents, and for the development of the diagnostic and therapeutic aspects of this Program Project.

**Grant:** 1P01AI055789-01A1  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** LIDDINGTON, ROBERT C PHD  
**Title:** Virulence Factors and Cell Death  
**Institution:** BURNHAM INSTITUTE LA JOLLA, CA  
**Project Period:** 2004/07/05-2009/06/30

DESCRIPTION (provided by applicant): Many virulence factors directly influence death pathways in infected hosts to counteract immune responses. In other instances, virulence factors delay cell death to allow sufficient time for pathogen replication, and ultimately induce cell death to aid in spread of the pathogen. This application seeks to define the link between several category A virulence factors and cell death (primarily apoptosis) pathways, and to design mechanism-based strategies to counteract the processes. The proposed Program Project consists of six projects and three supporting cores. In the first project, Dr. Liddington will determine crystal structures of key anthrax virulence complexes, as well as co-crystallize virulence factors in complex with inhibitors derived in the other projects and a Core. In the second project, Dr. Salvesen will determine targets and inhibitors of the Variola serpins. In the third project, Dr. Lipton will explore the neurodegenerative effects of botulinum toxin. In the fourth project, Dr. Reed will explore the function of novel virulence factors predicted by bioinformatics approaches. In the fifth project, Dr. Mustelin will explore the mechanism of cell death induced by Yersinia YopH. In the sixth project, Dr. Pellecchia will design and synthesize small molecules and peptides targeting high priority virulence factors. Two scientific Cores will provide the infrastructure for protein expression, purification and preliminary structural studies and high-throughput inhibitor screening, chemical synthesis and resynthesis of lead compounds. Each project has a discovery theme, and most have an applied component. The elements of the proposed Program Project are highly interdependent, with many complementary aims. The discovery themes range from elucidation of the mode of virulence factors binding to their targets, to the discovery of novel pathogen death regulators, to the design of peptide mimics and small molecules that interact with virulence factors. The applied components in most of the projects aim to discover peptide and small molecules that ablate virulence factor activity. As such, the latter project has a major impact on the applied components since it will produce molecules and strategies for testing in the other projects. Altogether, the information derived from these studies may reveal strategies for preventing or ameliorating cell death induced by pathogenic bacteria, thus complementing traditional antibiotics in the prevention and treatment of bacterial diseases and agents of biological warfare.

**Grant:** 1P01AI057699-01A1  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** KEARNEY, JOHN F PHD  
MICROBIOLOGY:IMMUNOLOGY  
**Title:** Immunity to Bacillus anthracis: Spore-Host Interactions  
**Institution:** UNIVERSITY OF ALABAMA AT BIRMINGHAM BIRMINGHAM, AL  
**Project Period:** 2004/07/01-2009/06/30

**DESCRIPTION** (provided by applicant): The overall objective of this program project is to provide sufficient understanding of the mechanisms of Bacillus anthracis spore-host interactions to facilitate the design and development of preventive, interventional and diagnostic procedures for the disease Anthrax. We will determine the detailed chemical structure of glycoproteins and other spore coat determinants and investigate the possibility that they can be used as new B. anthracis spore-specific targets for anthrax prevention and treatment. The detailed composition of the exosporium will be characterized by genetic and biochemical strategies. Individual mutations of all known, and to be discovered, exosporium components will be made. The structure of the carbohydrate components of the exosporium will be determined using a variety of genetic, chemical and spectroscopic methods. A mouse model will be used to identify the cellular pathways of spore entry and passage via the airways, gastrointestinal, cutaneous, and blood routes. Mice in which selected genes of the innate and adaptive immune system have been inactivated by gene-targeting will be used to define mechanisms of immunopathology and immune evasion of the ungerminated spores in the host. Wild type and mutant spores will be used to determine the role of these components in spore integrity, spore germination and survival in a mammalian host. We will isolate and characterize spore-receptors on host cells using spores, recombinant exosporium components and defined carbohydrates as ligands for these receptors. In addition, this project will also involve the construction of carbohydrate-protein conjugates and neoglycoproteins designed on the basis of our structural studies. These constructs, in addition to cloned and expressed recombinant exosporium proteins, will be used to enhance immune responses to the glycoconjugates on the exosporium of B. anthracis, and assist in the development of novel vaccine or interventional strategies that will optimize specific protective primary and secondary immune responses, and may lead to new strategies for blockade of spore entry into the host. Our overall strategy differs from that currently in use which is designed to induce protective immunity to the toxins elaborated by the vegetative form of B. anthracis after spore invasion and germination in the host. We will be able to identify potential mechanisms to rapidly inactivate spores prior to establishment of infectious loci and vegetative cell outgrowth. Therapeutic drugs of this nature would be a major supplement to the current recommended antibiotic regimens or in the case of multidrug-resistant B. anthracis strains engineered to produce additional toxins for use as bioweapons.

**Grant:** 1P01AI057836-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** MCNEIL, MICHAEL R PHD  
**Title:** MDR-TB Drugs: Targeting Cell Wall Synthetic Enzymes  
**Institution:** COLORADO STATE UNIVERSITY-FORT FORT COLLINS, CO  
COLLINS  
**Project Period:** 2004/06/15-2009/05/31

DESCRIPTION (provided by applicant): A purpose of NIH Notice NOT-AI-02-023 is to expedite research leading to the treatment of MDR-TB, a potential bioterrorism agent. New chemotherapies based on different targets than the present TB drugs are needed, but there is a paucity of the required leads ready for clinical trials. In response to this lack of required leads, it is proposed to develop new leads active against MDR-TB and effective in mice by developing inhibitors of several essential cell wall synthetic enzymes not targeted by current drugs. Compounds that inhibit three different cell wall synthetic enzymes (some of which also inhibit the growth of TB) have been identified by screening a chemical library with microtiter plate based enzyme assays. These and similarly identified hits will be refined into highly effective enzyme inhibitors also active against MDR-TB using a "compound refinement" cycle. The cycle begins with the "compound development group" doing X-ray crystallography studies of the targeted enzymes (especially enzymes with bond inhibitors), proceeding to molecular modeling to design new inhibitors and finally synthesizing a group of compounds based on this modeling and also incorporating chemical diversity. Then the "compound analysis group" determines efficacy of enzyme inhibition, MIC values on a panel of MDR-TB isolates, in vitro cell toxicity, and as warranted, toxicity in mice, efficacy in an interferon gamma knock out mouse, basic pharmacokinetics, and efficacy in a standard mouse model. The data from the compound analysis group is relayed to the compound development group so that a new round of further refined compounds can be prepared. The cycle is continued for each class of inhibitors until compounds with the desired properties emerge or it is determined that a particular class of compounds is unlikely to be yield new drugs. The cycle is presently ready to begin with inhibitors of three essential enzymes; in addition we will develop microtiter plate assays and screen chemical libraries for hits for four additional essential cell wall biosynthetic enzymes which will then enter the refinement cycle.



**Grant:** 2R01AI012202-31

**Program Director:** KORPELA, JUKKA K.

**Principal Investigator:** SOLL, DIETER G PHD CHEMISTRY:CHEMISTRY-UNSPEC

**Title:** PRINCIPLES OF SENSORY RECEPTION AND MOTOR FUNCTION

**Institution:** YALE UNIVERSITY NEW HAVEN, CT

**Project Period:** 1977/01/01-2007/01/31

DESCRIPTION (provided by applicant): This application asks how the flagellum of the bacterium *Salmonella* is assembled, and how most of its biomass, external to the cell, is exported. What is the composition and location of the export apparatus? How do its substrates cross the plane of the membrane? What does the assembly process entail? Is the export process ordered? If so, to what extent and what is the mechanism? How is the process energized and how is the energy transduced and controlled? The methods proposed include classical and molecular genetics (e.g., intergenic suppression and scanning deletion analysis), enzymatic biochemistry (characterization of the ATPase activity of the apparatus), and physical biochemistry (affinity chromatography, size filtration chromatography, etc.). A major emphasis will be on obtaining high-resolution structures of a number of the export apparatus components. This will initially be done with the thermophilic bacterium *Thermotoga maritima*, which has a similar export system but whose proteins are more stable and amenable to X-ray crystallography. These structural studies will be conducted in collaboration with Professor Namba of Osaka University, Japan. The application is one of basic microbiological research, but motility is often a significant contributing factor to pathogenicity. Also, Type III secretion pathways extremely similar to the flagellar protein export pathway are utilized by many pathogens (*Yersinia pestis*, for example) for secretion of virulence factors; information learned about one branch of this superfamily of pathways can only help with understanding of the other. Finally, the assembly of complex biological structures is one of broad general interest.

**Grant:** 2R01AI012575-29A1  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** ROTHMAN-DENES, LUCIA B  
**Title:** Novel Transcribing Activites in N4 Infected E. Coli  
**Institution:** UNIVERSITY OF CHICAGO CHICAGO, IL  
**Project Period:** 2004/07/01-2009/06/30

DESCRIPTION (provided by applicant): Our work focuses on the structure, mechanism of promoter recognition and activation of two phage N4-coded RNA polymerases, vRNAP and N4 RNAPII, which belong to the T7 RNAP-like family. The 3,500 amino acid vRNAP recognizes a hairpin and specific sequences at its promoters. Promoter activation requires supercoiling and EcoSSB. We defined and characterized an active central domain (1,106 mini-vRNAP, the most distantly related member of the family) and have recently determined its crystal structure at 2.0 Å resolution. We will define the in vivo structure of vRNAP promoters to support our model of supercoiled-induced hairpin extrusion, identify determinants of promoter recognition using biochemical and genetic approaches, determine the structure of the mini-vRNAP-promoter DNA complex by X-ray crystallography, define a nucleic acid scaffold for crystallization of the elongation complex, and define amino acid residues responsible for the EcoSSB-vRNAP interaction that elicits EcoSSB-assisted product displacement. N4 RNAPII is a heterodimer that does not recognize promoter sequences. In vivo it requires N4gp2, a ssDNA binding protein that recruits N4 RNAPII to ssDNA specifically. Middle promoters contain two sets of conserved sequences separated by 12-25 bp. We will identify all N4 RNAPII promoters and analyze their in vivo structure to test our model of promoter recognition, determine the crystal structure of N4 RNAPII and of its complex with gp2, characterize gp2 by determining its native MW, defining determinants of ssDNA-binding and of interaction with RNAPII, and the target of gp2 interaction in RNAPII. We will identify the N4-coded protein responsible for N4 RNAPII promoter specificity, and characterize its interaction with DNA, RNAPII and/or gp2 to reconstitute a system with purified components. We expect to provide new insights into strategies of promoter-RNAP interaction, into structure of factor-dependent T7-like RNA polymerases, and into the role of DNA structural transitions and single-stranded DNA binding proteins in transcription regulation.

**DESCRIPTION** (provided by applicant): This proposal is concerned with the molecular mechanisms that regulate the synthesis and degradation of membrane lipids in bacteria. The metabolism of two vitamins, lipoic acid and biotin, that are related to fatty acids are also studied. The general approach is to use genetics, biochemistry, and molecular biology to unravel these mechanisms and determine how the pathways are regulated. The mechanisms of fatty acid metabolism are highly conserved throughout metabolism thus giving these studies general applicability. However, in the case of fatty acid synthesis the details of the pathways differ sufficiently that compound that specifically inhibit the bacterial enzymes have been found. Therefore, bacterial fatty acid synthetic enzymes are excellent targets for new antibiotics. It should be noted that a group of protozoan parasites including the malarial parasite have essential fatty acid synthetic enzymes that are closely related to the bacterial proteins and thus new antibiotic should prove effective versus these parasitic diseases.

**Grant:** 2R01AI019497-18A1

**Program Director:** KORPELA, JUKKA K.

**Principal Investigator:** MALAMY, MICHAEL H PHD  
MICROBIOLOGY:MICROBIOLO  
GY-UNSPEC

**Title:** Genetic Systems to Study Virulence in Bacteroides

**Institution:** TUFTS UNIVERSITY BOSTON BOSTON, MA

**Project Period:** 1983/01/01-2009/04/30

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

**Grant:** 2R01AI020516-21  
**Program Director:** BOCEK, PETR  
**Principal Investigator:** SILVERSTEIN, SAMUEL C MD INTERNAL MED:INTERNAL  
MEDICINE-UNSPEC  
**Title:** Role of Mononuclear Leukocytes in Immunity  
**Institution:** COLUMBIA UNIVERSITY HEALTH SCIENCES NEW YORK, NY  
**Project Period:** 1984/07/01-2009/04/30

DESCRIPTION (provided by applicant): We have used three-dimensional fibrin and collagen I gels to compare neutrophil (PMN) bactericidal activity in tissue-like environments vs. stirred suspensions and discovered that a critical PMN concentration (CNC) is required to block the growth of bacteria in fibrin and collagen gels and in stirred suspensions. The concept that a critical leukocyte concentration is required to carry out specific immune effector functions is both novel and important. It unifies a large body of literature on host defense against bacterial infections, and provides a conceptual framework for assessing quantitatively the concentration of any class or type of immune cell that must be delivered to a tissue to execute a specific function. We have derived an equation that enables us to calculate the CNC for all bacterial concentrations under all experimental conditions so far tested. Using it we found the CNC in suspension (approximately  $4 \times 10^5$  PMN/ml), is almost identical to that known to predispose neutropenic humans to sepsis, while that in fibrin gels is 2.5 - 10-fold higher (e.g.,  $1-4 \times 10^6$  PMN/ml). Using these gels we have extended our previous observation that specific matrix proteins (e.g., fibrin) and chemoattractants (e.g., fMLP) block PMN migration by showing that in the presence of fibrin, fMLP blocks PMN bactericidal activity. Moreover, we discovered that PMN genetically deficient in  $\alpha 2$ -integrins phagocytose and kill *S. epidermidis* as efficiently as wild-type PMN in fibrin gels. We also have discovered that contrary to conventional wisdom, PMN have the capacity to invade and kill >98% of *S. epidermidis* in mature (5-day old) biofilms. We seek continued support to extend these studies, and to identify mechanisms that regulate emigration of PMN and monocytes from the blood into tissues, processes central to limiting tissue damage, and to delivering sufficient PMN and monocytes to eradicate planktonic and biofilm bacteria, and cytotoxic lymphocytes to kill cancer cells. This application has three Specific Aims. Aim #1. To measure the critical concentration of monocytes for killing *S. epidermidis* and *E. coli* in fibrin and collagen I gels in vitro, and the critical concentrations of PMN and of monocytes for killing of *S. epidermidis* and *E. coli* in vivo, and to identify the mechanisms that signal cessation of entry of PMN and of monocytes into dermal sites of bacterial infection. Aim #2. To identify the immunological mechanisms that impede PMN and monocyte killing of biomaterials-associated *S. epidermidis* biofilms. Aim #3. To determine whether the concept of a critical leukocyte concentration also applies to the tumoricidal activities of cytotoxic lymphocytes and monocytes. In other words, must cytotoxic lymphocytes reach a critical concentration within a tumor bed to effect a reduction in tumor mass?

**Grant:** 2R01AI021242-20  
**Program Director:** PERDUE, SAMUEL S.  
**Principal Investigator:** WALKER, DAVID H  
**Title:** Spotted Fever Rickettsial Antigens  
**Institution:** UNIVERSITY OF TEXAS MEDICAL BR GALVESTON, TX  
GALVESTON  
**Project Period:** 1992/09/30-2009/06/30

**DESCRIPTION** (provided by applicant): The long-term goal of this project is the elucidation of the mechanisms of protective immunity against spotted fever group rickettsiae. A strong framework of knowledge of the mechanisms of protective immunity that clear disseminated endothelial infection established by intravenous inoculation of *Rickettsia conorii* into susceptible C3H mice has identified an important gap in our knowledge, namely of the initial infection events. This competing renewal application proposes to close that gap by experiments focused on intradermal inoculation, rickettsial interactions with dendritic cells, T lymphocyte priming in the draining lymph nodes, dendritic cell-NK cell cross talk, and immunomodulatory effects of *Rhipicephalus sanguineus* tick saliva. Preliminary results indicate that *R. conorii* activates dendritic cells, which influence the outcome of infection. The specific aims are 1) Determine the differences between the responses of dendritic cells of inbred mice genetically resistant or susceptible to *Rickettsia conorii* infection when the dendritic cells (DCs) are activated by the rickettsiae in vitro and in vivo and the effects of the presence of tick saliva in the rickettsial inoculum on the rickettsia-DC interaction and 2) Determine the role of activation of DCs by *R. conorii* on the pathogenesis of spotted fever rickettsiosis in vivo, including the effects on innate and adaptive immunity, and the effects of tick saliva in the rickettsial inoculum on the anti-rickettsial immune response. The experimental design takes advantage of well-established mouse models including one with complete innate resistance, one with dose-dependent mortality caused by *R. conorii*, and one employing *R. australis* that causes dose-dependent mortality in C57BL/6 mice enabling use of gene knockout approaches. In vitro studies, flow cytometry, real time PCR measurement of rickettsial load, immunohistochemistry, cytokine assays, ELISPOT assays, CTL assays, and other immunological approaches will be utilized in critically designed experiments to determine the effective generation of anti-rickettsial immunity.

**Grant:** 2R01AI021678-19  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** GUNSALUS, ROBERT P  
**Title:** Anaerobic Expression of Fumarate Reductase in E. coli  
**Institution:** UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA  
**Project Period:** 1986/09/01-2009/04/30

DESCRIPTION (provided by applicant): The major aims of this research project are to further elucidate the regulatory mechanisms required for nitrate- and molybdate-dependent gene expression in Escherichia coli. This enteric bacterium is a model pathogenic and commensal enteric microorganism: it can detect nitrate and a variety of other respiratory substrates in the environment and respond accordingly by synthesizing different respiratory complexes in response to changing environmental signals. This strategy ensures optimal production of energy to support of cell maintenance reactions, biosynthesis, and cell growth. In the studies proposed herein, we will use several model nitrate/nitrite-controlled genes in this organism (e.g., fumarate reductase (frdABCD), NarG and NapF nitrate reductase (narGHJI, and napA) nitrite reductase (nrf and nirB), and DMSO/TMAO reductase (dmsABC) genes to examine how the alternative environmental signals are detected and how the resulting information is then utilized to modulate gene expression. Since these processes are common to many facultative animal and plant pathogens, the proposed molecular and biochemical studies should provide insight concerning how less well understood microbes manage the anaerobic transitions with respect to nitrate. We will examine the basis for nitrate, nitrite, and molybdate-dependent control of gene expression by the Nar and ModE regulatory circuits. We will extend our studies to further examine how the NarX, NarQ, NarP, and NarL proteins detect and transduce signals to modulate gene control. We will examine how the ModE protein provides for the molybdate-dependent control molybdate-containing enzymes to fine-tune expression of the anaerobic respiratory pathway genes. The Nar and ModE gene families will be identified by microarray analysis. Finally, the physiology of nitrate control will be examined by use of continuous culture methods.

**Grant:** 2R01AI022553-20  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** MODLIN, ROBERT L MD  
**Title:** Molecular analysis of host immune responses in leprosy  
**Institution:** UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA  
**Project Period:** 1991/01/01-2009/02/28

**DESCRIPTION** (provided by the applicant): The long-term objective of this proposal is to gain insight into mechanisms of cell-mediated immunity and unresponsiveness to intracellular pathogens in humans. Leprosy provides an extraordinary model to investigate these immunoregulatory processes. First, the disease primarily affects skin and is therefore accessible for study, proving a unique opportunity for investigating the nature of the local inflammatory response in human infectious disease. Secondly, the disease represents a spectrum of clinical manifestations that correlate with the pattern of cytokines produced at the site of infection. Patients with the resistant tuberculoid form (T-lep) express type 1 cytokines in lesions; whereas patients with progressive lepromatous form (L-lep) manifest type 2 cytokines in lesions. Our approach has been to study immune responses in lesions at the site of disease activity. Such studies have provided insight that cannot be obtained from the study of the peripheral blood from these patients. We hypothesize that the outcome of the host response to *M. leprae* is determined by the nature of the innate and adaptive immune response in lesions. We now propose to: 1) elucidate the gene expression profiles associated with resistance versus susceptibility in the human immune response to an intracellular pathogen, 2) determine the role of leukocyte immunoglobulin-like receptor (LIR) family members in contributing to immune unresponsiveness in leprosy; and, 3) elucidate the mechanism by which T-cell recognition of a major microbial antigen contributes to host defense in leprosy. The studies we propose are intended to provide a comprehensive analysis of the immune response in leprosy, comparing host responses in patients that are resistant to infection vs. susceptible to progressive disease. Such studies should provide new insights into mechanisms of immunoregulation in humans, and, we would hope, would provide the ability to predict disease outcome and also lead to the development of new immunomodulatory treatments for a variety of human infectious diseases. These issues are timely, given the rise in emerging infections and the threat of bioterrorist attacks.



**Grant:** 2R01AI023362-19  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** KRAUSE, DUNCAN C  
**Title:** Genetic Analysis of Mycoplasma Pneumoniae Cell Adherence  
**Institution:** UNIVERSITY OF GEORGIA ATHENS, GA  
**Project Period:** 1986/09/01-2009/04/30

DESCRIPTION (provided by applicant): Mycoplasma pneumoniae is the leading cause of pneumonia in older children and young adults. Fundamental aspects of mycoplasma cell and molecular biology are poorly understood, despite the significant impact of mycoplasmas on public health and agriculture. Development of more effective means of prevention and control requires a clearer understanding of the basic biological processes of these unique, cell wall-less prokaryotes. M. pneumoniae infections in humans are transmitted by aerosol, followed by the binding of mycoplasmas to the host mucosal blanket and respiratory epithelium. Adherence is mediated largely by a differentiated terminal organelle, a membrane-bound extension of the mycoplasma cell defined by the presence of an electron-dense core. Duplication of the attachment organelle precedes cell division. The focus of this proposal is the organization and assembly of the attachment organelle, with a long-term objective of identifying potential targets for more effective control of M. pneumoniae infections. Aim 1 will examine the structure of the attachment organelle in closer detail, defining the influence of protein HMW2 on the organization of the electron-dense core, examining protein-protein interactions by gel filtration and co-precipitation, and analysing the assembly process using green fluorescent protein fusions. Aim 2 will address the phenotype of a mutant lacking only the adhesin P1. Mycoplasma adherence will be assessed using polarized normal human bronchial epithelial cell monolayers. Recombinant derivatives of P1 will be engineered with epitope tags to establish membrane topography, and functional domains will be characterized through analysis of deletion derivatives. Aim 3 will examine the role of the MPN119 gene product in attachment organelle assembly. This protein shares with other novel cytoadherence-associated proteins an acidic proline-rich domain and a domain enriched in aromatic amino acids and glycine, but also has a J-domain, the signature motif of the HSP40 family of molecular chaperones. A non-cytoadhering mutant in which MPN119 has been insertionally inactivated by Tn4001 will be characterized in greater detail, including genetic complementation, identification and localization of the gene product, and evaluation of its role in attachment organelle assembly and function.

**Grant:** 2R01AI023549-19  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** SHUMAN, HOWARD A PHD  
**Title:** Genetic Analysis of Monocyte Killing by Bacteria  
**Institution:** COLUMBIA UNIVERSITY HEALTH SCIENCES NEW YORK, NY  
**Project Period:** 1986/06/01-2009/02/28

DESCRIPTION (provided by applicant): *Legionella pneumophila* is a gram-negative bacterial species that infects a wide range of phagocytic host cells, including human macrophages and unicellular protozoa. Infection of alveolar macrophages results in the acute pneumonia known as legionnaires' disease. Infection of protozoa in natural or man-made environments is thought to provide a reservoir of the organism during outbreaks of legionnaires' disease. *L. pneumophila* inhibits phagosome fusion with lysosomes to survive and modifies the phagosome so that it becomes a favorable site for replication. A Type IV secretion system called the Icm/Dot system is absolutely required for intracellular survival and replication in mammalian and protozoan hosts but is dispensable for growth on bacteriologic media. A generally accepted hypothesis is that the Icm/Dot system translocates several effector molecules to host cells and that these effectors are directly responsible for interacting with host cell components and modifying organelle trafficking. During this funding period we: (i) identified three *Legionella* effector proteins (LepA, B, and C); (ii) showed that LepA and LepB are required for release of *Legionella* from protozoan host cells; and (iii) demonstrated translocation and cell contact dependent secretion of the Leps via the Icm/Dot system. In order to understand the molecular basis of *L. pneumophila* intracellular multiplication, we will: identify additional effectors, characterize the properties of the Lep proteins, and identify the host proteins that interact with them. To accomplish these goals we propose to: (i) Identify *Legionella* effectors based on their ability to disrupt endosomal trafficking in yeast. (ii) Identify *Legionella* proteins that are translocated to eukaryotic cells via the Icm/Dot system. (iii) Determine which host cell proteins interact with *Legionella* effector proteins. (iv) Test the hypothesis that the IcmO protein is the coupling factor of the Icm/Dot System. (v) Determine if the coiled:coil regions of the Lep proteins are required for translocation by the Icm/Dot system and/or for their function in host cells.

**Grant:** 2R01AI024616-16  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** APICELLA, MICHAEL A MD INTERNAL MED:INTERNAL  
MEDICINE-UNSPEC  
**Title:** H. influenzae: LOS and biofilms in pathogenesis  
**Institution:** UNIVERSITY OF IOWA IOWA CITY, IA  
**Project Period:** 1988/03/01-2009/02/28

DESCRIPTION (provided by applicant): Nontypeable *Haemophilus influenzae* (NTHi) commonly colonize the mucosal surfaces of the human upper respiratory tract. For the most part, this species resides in that environment in a benign (symbiotic?) relationship with the human host. With pathophysiologic changes (obstruction of outflow ducts, loss of the mucociliary elevator, viral infection, etc.) in the airway anatomy and mucosal surface, NTHi can cause human upper and lower tract respiratory infection. Recent studies in our laboratory using established assays suggest that NTHi produces a biofilm and that a CMP-NANA synthase (*siaB*) and an ( $\alpha$ 2-3 sialyltransferase (*siaA*) mutant cannot make a biofilm. Our studies have also shown that both mutants can initially adhere to human bronchial epithelial cells as well as the NTHi 2019 parent strain. In addition, a mutation in another glycosyltransferase, *Rfe*, which is a homologue of an undecaprenyl-phosphate  $\alpha$ -N-acetylglucosaminyltransferase, produces no biofilm. In addition, growth of NTHi 2019 in sialic acid deficient defined media significantly reduces biofilm formation. A mutation in *lsgG*, a homolog of the regulator, *Escherichia coli* *modE*, also produces a reduced amount of biofilm. Formation. Our studies would suggest that NTHi makes a biofilm matrix in part composed of sialic acid and lacks glucose and galactose. Based on these observations, we propose to explore the factors controlling the production of biofilm formation during NTHi infection of bronchial epithelia, the impact of biofilm formation on LOS expression and interactions between NTHi and primary human bronchial epithelial cells during long-term infections. The hypotheses underlying this proposal are 1) that NTHi exists in a biofilm during bronchial infection and that this capability is under the control of a bacterial or environmental signal 2) that the NTHi biofilm is either entirely or predominately composed of carbohydrate, likely containing sialic acid and 3) that gene and protein expression of NTHi in the biofilm and planktonic phase are different, and 4) that there is a linkage between LOS expression and biofilm formation. To examine these hypotheses, we would propose the following Specific Aims: 1) The elucidation of the structure of the NTHi biofilm and the genes responsible for its biosynthesis, 2) The determination of the genes involved in biofilm biosynthesis and the differences in gene and protein expression in biofilm and planktonic NTHi and 3) The molecular analysis of the effects of prolonged NTHi infection on primary human bronchial epithelial cells.

**Grant:** 2R01AI025567-13  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** MOBLEY, HARRY L PHD MICROBIOLOGY, OTHER  
**Title:** Urease and Gene Expression of Helicobacter Pylori  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 1989/08/01-2009/06/30

**DESCRIPTION** (provided by applicant): Peptic ulcer disease is a common malady in the United States affecting up to 10% of men and 4% of women over their lifetimes. With manifestations such as abdominal pain, nausea, and vomiting, 500,000 new cases of duodenal ulcer and 100,000 new cases of gastric ulcer are diagnosed each year. Sequelae include gastric cancer. Helicobacter pylori, a gram-negative, microaerophilic spiral-shaped bacterium is the most frequently cited etiologic agent of human gastritis and peptic ulceration. This species, whose niche is highly restricted to the gastric mucosa of humans, has adopted a strategy of survival that includes synthesis of urease as its most abundant cellular protein. Nickel ions are now recognized as a critical cofactor required for catalytic activity of urease and are necessary for induction of certain H. pylori genes and repression of others. We propose that this ion serves as an important environmental cue in the gastric mucosa that up-regulates H. pylori genes needed for colonization of this hostile niche. Nickel acquisition, mediated in part by the 8-transmembrane domain-NixA nickel transport protein, in H. pylori could be viewed as important as is iron acquisition for other bacterial pathogens. In the current proposal, we hypothesize that a network of ion transport, ion-binding, and ion-chaperoning proteins modulate urease activity and virulence of H. pylori. To test this hypothesis, we propose as Specific Aims to use molecular genetic, genomic, proteomic, and bacterial physiological methodology to: 1) Identify genes up-regulated in response to nickel ion limitation in vitro; 2) Determine the transcriptome of H. pylori in vivo and the effect of pH change and nickel limitation; and 3) Correlate gene expression of ion homeostasis genes with urease synthesis, urease activity and nickel insertion into the urease apoenzyme. The transcriptome of H. pylori 26695 and selected mutants, cultured in vitro and in vivo, will be quantified using H. pylori-specific macroarrays. Genomic results will be validated by kinetic RT-PCR. Newly discovered ion homeostasis genes will be subjected to mutagenesis and virulence studies in the Mongolian gerbil model of infection. Alternative methods will include 2-D Fluorescence Difference Gel Electrophoresis. The long-term goals of the study are to understand the mechanism by which H. pylori can colonize the gastric mucosa and to identify more effective targets for therapeutic intervention.

**Grant:** 2R01AI029471-13

**Program Director:** CASSELS, FREDERICK J.

**Principal Investigator:** LEVINE, MYRON M MD OTHER CL MED:CLINICAL  
MEDICINE,UNSPEC

**Title:** Recombinant & live oral Salmonella typhi hybrid vaccines

**Institution:** UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD  
SCHOOL

**Project Period:** 1990/04/01-2009/02/28

DESCRIPTION (provided by applicant): In November 2002 in China, an outbreak of atypical pneumonia occurred in which a proportion of cases were very severe or fatal, and a high lethality was seen among elderly patients. The clinical syndrome began with fever, dry cough, myalgia and sore throat and progressed to atypical pneumonia. Outbreaks followed thereafter in 2003 in Vietnam, Hong Kong, Singapore, Canada, and Taiwan. Extraordinary characteristics of this global epidemic of "Severe Acute Respiratory Syndrome" (SARS) include the rapid isolation of the etiologic agent (a novel coronavirus; SARS-CoV), elucidation of the complete sequence of the viral genome, accelerated development of diagnostic tests, and rapid global exchange of clinical, epidemiologic and microbiologic information via the Internet by scientists and health officials in many countries. Investigators in the USA and Hong Kong were first to isolate from patients the novel coronavirus that is distinct from previously recognized groups of coronavirus. The underlying hypothesis of this research plan is that by appropriate manipulation of attenuated *Salmonella enterica* serovar Typhi (S. Typhi) and *Shigella* live vectors it will be possible to develop a mucosally-administered "prime-boost" vaccination strategy to prevent SARS. We will utilize attenuated S. Typhi or *Shigella flexneri* 2a live vector vaccine strains to deliver (via mucosal immunization) a Sindbis eukaryotic DNA replicon encoding the S (spike) and M (membrane) glycoproteins and the N nucleocapsid protein of the Urbani strain of the SARS-CoV to prime the immune system to recognize these coronavirus antigens. We will then boost the immune response by mucosally administering proteosomes (meningococcal outer membrane protein vesicles) to which the same SARS proteins are adsorbed (along with a lipopolysaccharide adjuvant). Virus-like Particles and attenuated S. Typhi expressing SARS peptide epitopes will serve as back-up boosting strategies. We will study whether these constructs can elicit the relevant immune responses, first in mice, then in cynomolgus monkeys, and finally in clinical trials in humans (the latter under separate funding). The induction of B and T cell memory pools will also be examined in monkeys. This approach aims to mimic the strong and broad immunity elicited by live virus vaccines with the inherent safety factor of not having to use putative attenuated live SARS virus derivatives. If the proposed vaccination strategy can indeed elicit broad, balanced and long-lasting immune responses in cynomolgus monkeys, these studies can be followed by a challenge (under respiratory pathogen biosafety level 3 containment) to assess the efficacy of the vaccine against wild type SARS-CoV.

**Grant:** 2R01AI031088-10A2  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** ARMSTRONG, SANDRA K PHD  
**Title:** Iron acquisition in *Bordetella pertussis*  
**Institution:** UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN  
**Project Period:** 1991/08/01-2008/11/30

**DESCRIPTION** (provided by applicant): *Bordetella pertussis* is the bacterial agent that colonizes the human respiratory epithelium to cause whooping cough. To obtain nutritional iron, *B. pertussis* produces the siderophore alcaligin and also expresses activities required for utilization of host heme compounds as well as certain non-native siderophores, including the potent siderophore enterobactin. These three iron-retrieval systems have distinct positive transcriptional regulators that respond to the cognate iron source for maximal expression of the genes required for its utilization. The ability of *Bordetella* cells to selectively express relevant scavenging systems for available iron sources may be important for effective adaptation and multiplication in the host environment. These studies will evaluate the humoral immune response of infected hosts to *Bordetella* iron system receptors and assess the *in vivo* importance of each of the three iron uptake systems in animal models of infection. Mechanistic features of siderophore signaling and transcriptional activation will be delineated for the alcaligin siderophore system and the enterobactin siderophore utilization system. The importance of the ability to transcriptionally respond to the appropriate iron source *in vivo* will be evaluated using *Bordetella* mutants producing novel hybrid regulators with reversed inducer and target gene specificities. A cell surface signaling phenomenon uniquely involved in regulation of the *Bordetella* host heme-iron utilization system will be investigated, and interacting signaling and regulatory protein domains will be defined. Spatiotemporal analysis of *in vivo* expression of the three iron systems will determine which systems are operational in the animal host and assess whether the systems are differentially expressed in certain tissue sites or during distinct stages of infection. Because *B. pertussis* is an obligate human pathogen with no known environmental or nonhuman animal reservoirs, it represents an ideal model organism for analysis of the host-parasite relationship and the physical, chemical and innate biological conditions that impact on the growth of bacteria in a host environment. This project will provide a better understanding of *Bordetella pertussis* pathogenic mechanisms and the infection process, as well as host immune responses involved in clearance and protection.

**Grant:** 2R01AI032178-11  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** GUINEY, DONALD G MD MEDICINE  
**Title:** Plasmid-Mediated Virulence in Salmonella  
**Institution:** UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA  
**Project Period:** 1993/09/01-2008/11/30

**DESCRIPTION** (provided by applicant): The long-term goal of this project is to define bacterial virulence factors and host responses that determine the outcome of serious, disseminated Salmonella infections. Non-typhoid Salmonella strains are common causes of food-borne illness in the United States and have been used effectively in a bioterrorism attack in this country. In addition, Salmonella represent an important research tool to study the molecular biology and immunology of the host-pathogen interaction. This project focuses on the Salmonella *spv* locus, a critical virulence determinant required for systemic disease. The *spv* operon encodes SpvB, an ADP-ribosyl transferase that modifies actin monomers in infected cells and leads to loss of the actin cytoskeleton. The active NAD-binding site of SpvB is located in the C-terminal domain and is required for the virulence phenotype. SpvB is the first ADP-ribosylating toxin that acts specifically from bacteria located intracellularly, and the first actin modifying toxin shown to be crucial for intracellular pathogenesis. This project will focus on the novel aspects of SpvB secretion and transport that allow the toxin to access the host cell from the phagosome rather than from the extracellular environment. The mechanisms of cytotoxicity and cell death mediated by SpvB will be determined. The Specific Aims are: 1) to analyze the mechanism of SpvB secretion and transport from the phagosome into the host cell cytoplasm. The hypothesis is that this process requires both the SPI2-encoded bacterial type III secretion system and specific regions of the SpvB protein. 2) to define the role and function of the N-terminal domain of SpvB in intracellular infection. The hypothesis is that the N-terminal domain contains regions that specify transport from the phagosome into the host-cell cytoplasm. 3) to determine the pathophysiologic consequences of actin depolymerization in the host cell during intracellular infection by Salmonella. The hypothesis is that actin depolymerization disrupts multiple aspects of cellular function, enhances intracellular growth, and triggers a delayed cell death pathway that enhances the cell-to cell spread of Salmonella during the infectious process.

**Grant:** 2R01AI033537-11  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** KOEHLER, THERESA M. PHD  
**Title:** Virulence Gene Expression by *Bacillus anthracis*  
**Institution:** UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX  
HOUSTON  
**Project Period:** 1992/12/01-2008/11/30

DESCRIPTION (provided by applicant): *Bacillus anthracis*, a Gram-positive spore-forming soil bacterium and member of the *Bacillus cereus* group species, is distinguished by its ability to cause lethal anthrax disease in mammals, including humans. Well-established virulence factors unique to this organism are the anthrax toxin proteins and a poly-D-glutamic acid capsule. Findings of numerous investigators have established the structure and function of the anthrax toxins and capsule. Work in our laboratory has focused on the genetic basis for expression of the structural genes for the toxin proteins, *pagA*, *lef*, and *cya*, and more recently, the capsule biosynthesis operon, *capBCAD*. Our model for virulence gene regulation is of increasing complexity and includes numerous trans-acting regulators. The most critical and far-reaching regulator is *atxA*, a gene that appears to be unique to *B. anthracis*, *atxA* is essential for expression of all three toxin genes and contributes to control of the capsule operon. In experiments proposed here, we will continue our investigations of virulence gene expression by testing our current model for regulation of virulence. Our overall approach will be to determine the function of virulence gene regulators in *B. anthracis* cultured in vitro and to test for significance in a mouse inhalation model for anthrax. We will also assess the physiological roles of newly identified targets of established regulators. Finally, we will probe the molecular basis for differences in beta-lactamase gene expression between prototypical penicillin-susceptible and less common penicillin-resistant *B. anthracis* strains. *Bacillus anthracis* is the lead bacterium on the Select Agent List. The intentional release of spores in the fall of 2001 in the U.S. that resulted in eleven confirmed cases of anthrax and five deaths dramatically illustrated the public health threat this organism can pose when as a bioweapon. As the recent U.S. cases showed, inhalation of *B. anthracis* spores can result in a fatal clinical outcome in humans and only timely post-exposure intervention can limit the extent of the disease. Our overall objective is to identify and characterize *B. anthracis* determinants that impact *B. anthracis* infection in a mouse model for inhalation anthrax. Such determinants are potential targets for therapeutic intervention and/or possible components for new subunit vaccines.



**Grant:** 2R01AI035705-11  
**Program Director:** PETERS, N KENT  
**Principal Investigator:** ARCHER, GORDON L MD INTERNAL MED:RENAL DISEASES  
**Title:** Staphylococcal methicillin resistance locus  
**Institution:** VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA  
**Project Period:** 1994/04/01-2008/12/31

DESCRIPTION (provided by applicant): Staphylococci are the leading cause of hospital-acquired infections, especially nosocomial bacteremia. The two most effective and widely used anti-staphylococcal therapeutic agents are glycopeptides and beta-lactams, both of which target cell wall biosynthesis. However, therapy with these agents is becoming less effective as resistance has developed, first to beta-lactams and, more recently, to glycopeptides. The most important mechanism of resistance to beta-lactams is the acquisition of a new target, a cell wall transpeptidase or penicillin binding protein (PBP2a) that is not inactivated by the antibiotic. This type is called methicillin or oxacillin resistance (OR) and the gene that mediates this resistance, *mecA*, is encoded within a pathogenicity island called SCCmec. The following proposal seeks to continue studies that explore the origin, dissemination and regulation of genes that mediate OR and genomic adaptations required for staphylococci to become resistant to agents that damage their cell walls. The First Specific Aim will be to investigate the transfer of SCCmec between strains of *Staphylococcus aureus* (SA) and from a different staphylococcal species, *S. epidermidis* (SE), to SA. There is evidence that a new SCCmec type, Type IV, has recently moved into SA isolates prevalent in the community and it is present in the majority of SE isolates. The excision, transfer (by plasmid and phage) and reinsertion of this element will be investigated. The Second Specific Aim will be to continue studies on the induction of *mecA* transcription through the sensor/transducer, MecR1, resulting in the release of the transcriptional repressor, MecI, from its DNA binding site. The basis of signal transduction and role of proteolytic cleavage of inducer and repressor will be assessed by constructing chimeric molecules, determining the crystal structure of repressors and identifying additional chromosomal genes required for induction. The Third Specific Aim will be to confirm and expand observations made by microarray transcriptional profiling that purine biosynthesis is altered in strains that develop high level resistance to vancomycin and oxacillin, but in opposite directions (increased and decreased respectively). These two phenotypes appear to be mutually exclusive. The purine biosynthetic operons will be genetically manipulated and correlated with development of VR and OR. In addition, microarray and proteomic studies will be pursued on other agents that perturb the cell wall.

**Grant:** 2R01AI035875-12  
**Program Director:** BOCEK, PETR  
**Principal Investigator:** RAVETCH, JEFFREY V MD INTERNAL MED:INTERNAL  
MEDICINE-UNSPEC  
**Title:** FcR Deficient Mice Susceptibility to Pathogens  
**Institution:** ROCKEFELLER UNIVERSITY NEW YORK, NY  
**Project Period:** 1994/05/01-2009/01/31

**DESCRIPTION** (provided by applicant): The host response to blood borne pathogenic organisms involves the responses facilitated by the marginal zone where pathogens first come in contact with cells of the immune system. The antibody response elicited by the marginal zone upon exposure to encapsulated microorganisms is dominated by the mouse IgG3 subclass in a T independent and complement C3 dependent reaction. Our work has focused on the genetic pathways that contribute to the organization of the marginal zone and the cellular responses that are triggered by this anatomic structure upon exposure to encapsulated microbial pathogens. Two main areas of investigation will be pursued in this proposal for the next granting period - 1) characterization of a novel FcgammaR that engages IgG3, called FcgammaRIV, and its role in the host response to encapsulated microbial pathogens and 2) dissection of the mechanisms that govern the coordinated movement of marginal zone B cells and macrophages in response to microbial challenge through the analysis of specific genetic pathways, such as *pyk2* and *SHIP*, that we have identified as being critical in the organization and function of the marginal zone. To accomplish these broad goals, four specific aims will be addressed: 1) Define the structure, function and expression of FcgammaRIV on myeloid and lymphoid cells. Experiments are proposed to define the expression and regulation on various cell types, the structural basis for its IgG3 binding specificity, the subunit composition and signaling properties of the receptor and its interactions with complement pathways and inhibitory signaling responses. 2) Analyze the *in vivo* role of FcgammaRIV in host response to encapsulated pathogens. Mice with conditional deficiency of FcgammaRIV in either lymphoid or myeloid cells will be constructed, combined with similar deficiencies in FcgammaRI, II or III and studied in both thymic dependent and thymic independent responses. Passive or active pathogen specific anti-capsular polysaccharide responses will be generated in these strains and the resulting animals challenged with *S. pneumoniae* and *C. neoformans* to determine the role of specific FcRs in protection or enhancement. 3) Characterize the molecular interactions between marginal zone macrophages and marginal zone B cells. The endogenous ligand for the MARCO scavenger receptor on marginal zone B cells will be identified and characterized for its expression and function *in vitro* and *in vivo*. The role of this receptor/ligand pair in marginal zone organization and response to capsular polysaccharides will be pursued. 4) Determine the mechanism of cellular migration in the marginal zone in response to microbial challenge. *Pyk2* and *SHIP* conditional mutants will be analyzed for their role in marginal zone B cell migration, retention and survival. The role of specific macrophage subsets will be investigated in the B cell and macrophage movement triggered by *S. pneumoniae* and *S. aureus*. These studies will clarify the host response to encapsulated microbial pathogens through the analysis of the marginal zone and specific antibody response elicited by these pathogens.

**Grant:** 2R01AI035950-12  
**Program Director:** DONG, GANG  
**Principal Investigator:** SWANSON, JOEL A  
**Title:** Manipulation of Phagocytosis in Macrophage  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 1994/05/01-2009/02/28

**DESCRIPTION** (provided by applicant): The long range goals of this research are to understand the microbial mechanisms of activated macrophages and to devise methods for therapeutic manipulation of infections. The experimental model for these studies is in vitro infection of murine macrophages with the bacterium *Listeria monocytogenes* (Lm). In non-activated, J774 or bone marrow-derived macrophages, bacteria escape from vacuoles into cytoplasm shortly after they are internalized by phagocytosis. Macrophages activated with interferon- $\gamma$  plus lipopolysaccharide inhibit growth of internalized bacteria by preventing their escape from the vacuole. The rapid escape and its direct inhibition by the activated macrophage provide a functional setting for identification of essential microbicidal chemistries inside activated macrophages. The hypotheses to be tested are that escape of Lm into cytoplasm of activated macrophages is inhibited by altered membrane trafficking and delivery into the vacuole of reactive oxygen and reactive nitrogen intermediates that inhibit perforation; and that these activities are coordinated by the controlled recruitment of the GTPases Rab5a and Rac2. This research project will determine how activation changes macrophage vacuolar compartments, by applying quantitative microscopic methods for measuring intracellular chemistries. The timing of vacuole and phagosome maturation in activated and non-activated macrophages will be quantified using time-lapse, ratiometric fluorescence microscopy of live, Lm-infected macrophages. The association of various fluorescent organelle markers with vacuoles containing wild-type or mutant Lm or with phagosomes containing opsonized erythrocytes will be quantified. Markers will include chimeras of citrine (a variant of YFP) plus actin, Rab5a, Rab7, LAMP-1, 3 phosphoinositide-binding domains, Rac1, Rac2, inducible nitric oxide synthase (iNOS), and gp47 phox, a component of the phagocyte oxidase complex. Fluorescence microscopy will be used to localize cholesterol. Vacuoles perforated by Lm will be identified using new methods for detecting bacterial escape into cytoplasm. Reactive oxygen intermediate (ROI) and reactive nitrogen intermediate (RNI) generation in vacuoles will be localized relative to vacuole maturation, perforation and bacterial escape. To identify signaling complexes, fluorescence resonance energy transfer (FRET) microscopy will be used to localize activated Rac1 and Rac2, and to detect interactions between those proteins and iNOS, gp47phox and Rab GTPases on vacuoles. FRET stoichiometry will be applied to measure relative concentrations of fluorescent chimeras on phagosomes and Lm vacuoles. The contributions of Rab5a and Rac2 to phagosome maturation and prevention of Lm escape in activated macrophages will be measured in cells expressing mutant forms of those molecules, including dominant negative or constitutively active Rac1, Rac2 and Rab5a, and in cells depleted of Rac2 via small inhibitory RNAs. Consequent effects on the efficiency of Lm escape from vacuoles will be quantified.

**Grant:** 2R01AI037139-07A1  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** CHATTERJEE, DELPHI PHD  
**Title:** Structure of LAM in Relation to Biology and Biosynthesis  
**Institution:** COLORADO STATE UNIVERSITY-FORT FORT COLLINS, CO  
COLLINS  
**Project Period:** 1996/06/01-2009/02/28

**DESCRIPTION** (provided by applicant): The re-emergence of tuberculosis as a public health problem has been complicated by the lack of effective chemotherapeutic agents and the development of drug-resistant strains. The cell wall of the pathogen *Mycobacterium tuberculosis*, is known to be the target of some of the most effective anti-mycobacterial drugs including ethambutol which is known to inhibit the biosynthesis of the arabinan of cell wall arabinogalactan (AG) and the associated lipoarabinomannan (LAM). A diverse range of biological studies over the past few years has collectively provided compelling evidence implicating LAM as a key surface molecule in host-pathogen interactions. The availability of truncated, mutated LAM variants as a consequence of drug resistance and genetic manipulation provides invaluable model compounds for both structural and functional studies aiming at defining the relevance of LAM in pathogenesis. Specifically, the fine details of the arabinan assembly and its point(s) of attachment to the phosphatidylinositol mannan core will be characterized and structural niceties positively correlating with particular biological attributes of clinical isolates will be identified. Structural basis of microheterogeneity in LAM will be defined and chemically and/or enzymatically modified arabinan and mannan will be derived from LAM for structural/biological studies. As a major spin off, the recent availability of the consequential cell wall mutants due to genetic manipulation of the embCAB proteins, and analyses of the gene products now allows us rationally to dissect the pathway to the formation of the arabinan of LAM/AG. In the same vein, studies on LAM mutants in *M. tuberculosis* and our concerted efforts on generating LAM depleted *M. tuberculosis* will contribute directly into addressing the role of LAM in survival/infectivity of the organism. Thus, the unifying theme of this Research Proposal encompasses structural analysis and manipulation of LAM, supplemented by genetic probes to alter its structure and mutate LAM in *M. smegmatis* and *M. tuberculosis* all in relation to biology and biosynthesis.

**Grant:** 2R01AI037856-06A1  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** BISHAI, WILLIAM R MD  
**Title:** M. tb. Survival Regulatory Genes  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 1997/04/01-2008/12/31

DESCRIPTION (provided by applicant): Mycobacterium tuberculosis is a leading AIDS-related infectious disease killer worldwide. Tuberculosis is a disease of multiple pathologic stages, and hence M. tb. must possess multiple adaptive genetic strategies to survive in these differing environments. Understanding the organism's pathogenesis mechanisms during these disease stages is the surest way to develop better diagnostics, vaccines, and drugs which are critically needed for patients with TB and TB/HIV. We have genetically interrupted 5 M. tb. sigma factor genes, identified the genes which they regulate by using microarray technology and assessed their virulence in mice. Among these alternative sigma factors there is functional redundancy in that upon infection of mice 4 of the 5 mutants show the immunopathology phenotype of attenuation in which mycobacterial counts are maintained at high level but there is a significant delay in mortality and in disease progression in the lungs. In the first aim, we will explore the immunopathology defect demonstrated by several of the sigma factor knockout mutants. The roles of known mediators of TB control such as nitric oxide, TNF-alpha, interferon-gamma and phagocyte oxidase will be examined using the M. tb. deltasigH and other knockout mutants which display the immunopathology phenotype. Second, we will refine our understanding of these sigma factor regulons by studying expression profiles under stress conditions, by biochemical analysis of transcription, by constructing double knockout mutants, and through conditional expression of sigma factors. We will address whether there is sufficient ECF promoter consensus degeneracy to permit redundant sigma factor control of dependent genes or whether there are distal mediators which remain to be discovered. Finally, in the 3rd aim we will explore the modulation of sigma factor activity by studying anti-sigma factors. We will study the effect of AsiA, a phage-encoded anti-sigma factor which binds to RNA polymerase, remodels it, and alters its promoter specificity. We will seek to determine whether AsiA or portions of it have a transcription-specificity modifying effect in mycobacteria. We will also study the role of a novel sigma factor-regulator in M. tb, Rv1364c, which our data show is required for resistance to SDS stress. Advancing our understanding of M. tb sigma factors and their related regulators will help establish key adaptive mechanisms in the pathogenesis of TB.

**Grant:** 2R01AI038396-07A1  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** WILSON, BRENDA A  
**Title:** Pasteurella multocida toxin: Structure and Activity  
**Institution:** UNIVERSITY OF ILLINOIS URBANA- CHAMPAIGN, IL  
CHAMPAIGN  
**Project Period:** 1996/08/01-2009/02/28

**DESCRIPTION** (provided by applicant): Pasteurella multocida toxin (PMT) is a major virulence factor associated with progressive atrophic rhinitis, respiratory disease in animals, and dermonecrosis, respiratory disease, and bacteremia in humans resulting from bite wounds or exposure to infected animals. PMT is a 1285 amino acid protein that can act on multiple cell types. It enters mammalian cells via receptor-mediated endocytosis and activates intracellular signal transduction events, including phospholipid hydrolysis, calcium mobilization, protein phosphorylation, DNA synthesis, and cytoskeletal rearrangements, which cause cell proliferation. We have demonstrated that the PMT-mediated stimulation of phospholipase C activity occurs through transient, but irreversible PMT action on the Gq protein. We have proposed a model for PMT action. We also characterized a number of the Gq-dependent pathways using PMT, and our results have led us to hypothesize that the pleiotropic effects of PMT on different cells is due to the diverse roles that the Gq target plays in the different cell types. In addition, we determined that the N-terminus of PMT is important for intracellular activity and that both N- and C-termini are important for binding and entry into mammalian cells. Our hypothesis is that PMT entry is mediated through multiple binding determinants on the toxin protein and through multiple receptors. Our long-range goals are to understand the structure and mechanism of action of PMT at the molecular and biochemical level, to facilitate future therapeutic intervention in *P. multocida* disease and to increase our preparedness against potential bacterial toxin-related threats involving similar mechanisms, as well as to increase our understanding of the molecular signaling events involved in Gq-dependent signaling. To achieve our goals, we propose the following Aims: (1) To elucidate the molecular mechanism by which PMT acts on the Gq-protein, by determining whether the effect of PMT on Gq-protein is caused by covalent modification or by direct or indirect protein interaction. (2) To determine the biochemical basis for the effect of PMT on Gq-coupled signal transduction, (I) by determining the effect of PMT on Gq activity and (II) by determining the effect of PMT on downstream Gq-signaling pathways. (3) To define the functional domain(s) of PMT responsible for binding eukaryotic cell receptors and translocating the intracellular activity domain into the cytosol. (4) To characterize the cellular receptor(s) and to elucidate the internalization pathway(s) utilized by PMT to gain entry into cells.

**Grant:** 2R01AI040305-06A1  
**Program Director:** MALLIA, CONRAD M.  
**Principal Investigator:** SILVERMAN, GREGG J MD  
**Title:** B Cell Superantigens and Immune Regulation  
**Institution:** UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA  
**Project Period:** 1998/05/01-2009/01/31

DESCRIPTION (provided by applicant): Superantigens (SAGs) for B lymphocytes interact via conserved V region framework sites in the B cell antigen receptor (BCR) to target large sets of lymphocytes. We have previously elucidated central structural and immunobiologic properties of protein A of *Staphylococcus aureus* (SpA), and established SpA as the prototypic experimental B-cell superantigen. Based on an understanding of the molecular basis by which naturally pentameric SpA binds B-cells, we have recently developed the murine T15i Ig "knockin" system for investigations of the in vivo outcome of SpA exposure. In these mice, most B cells express a VH transgene product that is targeted by SpA, and we have shown that SpA treatments rapidly induce activation-associated apoptotic death of targeted B cells. In the current research program, we will use different forms of SpA to elucidate key mechanisms responsible for BCR-mediated determinations of lymphocyte clonal fate. The Specific Aims will include: AIM 1: To define the nature of the SAG-induced BCR complex responsible for B lymphocyte activation and apoptosis. AIM 2: To determine how membrane co-receptors may affect clonal fate after interactions with SpA. AIM 3: To investigate how Bcl-2 family members may be involved in determining B-cell clonal fate after interactions with SpA. AIM 4: To evaluate how co-exposure to other immunologically active components of *S. aureus* can affect the outcome of in vivo SpA exposure. These investigations will provide important insights into the fundamental properties of B-cells. In addition, these studies will lead to a better understanding of the immunobiologic activities of a virulence factor from one of the most important causes of life-threatening infection in the US.

**Grant:** 2R01AI040588-05A1  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** SMITH, CHARLES J PHD MICROBIOLOGY, OTHER  
**Title:** ROLE OF B.FRAGILIS OXYGEN STRESS RESPONSE IN INFECTION  
**Institution:** EAST CAROLINA UNIVERSITY GREENVILLE, NC  
**Project Period:** 1998/12/01-2008/11/30

DESCRIPTION (provided by applicant): The anaerobic organism, *Bacteroides fragilis* is exceptionally resistant to the toxic effects of oxygen. This resistance can be attributed to induction of an oxidative stress response (OSR) and this response will be studied to document its role in the pathogenesis of *Bacteroides* infections. It is expected that new mechanisms of free radical protection and novel antioxidant defense strategies will be uncovered. This idea is supported by the fact that *B. fragilis* has diverged early from other eubacteria and has independently evolved the ability to survive extended exposure to oxygen. Further, since this is an anaerobe it is likely to have stringent requirements for oxygen protection that have resulted in the development of unique highly protective antioxidants. The long-term goals of this research are to understand the basic physiological and genetic processes responsible for *B. fragilis* oxygen tolerance and how these contribute to virulence. Specific objectives for this proposal are: 1) Delineate novel mechanisms that regulate expression and function of OxyR. OxyR is a redox sensitive transcriptional regulator that governs expression of the OSR. This aim proposes to pursue analysis of unique mechanisms through which OxyR regulates genes expression. In addition we will test the hypothesis that the *B. fragilis* thioredoxin system is the predominant system for maintaining the cellular redox balance and as such it controls the redox state of oxidative stress responsive regulatory proteins such as OxyR. 2) Characterize the role of Fur homologs in control of the OSR. This aim will test the hypothesis that the three Fur homologs in *B. fragilis* are required for protection during oxidative stress due to their regulation of metal (Fe) homeostasis and other OSR genes. 3) Elucidate a role for the OSR in *B. fragilis* infections. This aim will address the hypothesis that the OSR is necessary for initiation of infection and for persistence in the host. Our approach is composed of two phases that will examine the effect of OSR mutations on survival in animal models that mimic the two different stages of infection. Understanding oxygen tolerance may lead to identification of new antibiotic targets effective for treatment of *B. fragilis* infections. For example, exclusive dependence on thioredoxins for redox control may be a weakness that can be exploited by drugs targeted against thioredoxin reductase.



**Grant:** 2R01AI040662-05A2  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** LEVIN, BRUCE R PHD  
**Title:** Population Genetics & Evolution of Antibiotic Resistance  
**Institution:** EMORY UNIVERSITY ATLANTA, GA  
**Project Period:** 1997/08/01-2009/04/30

DESCRIPTION (provided by applicant): Acquired antibiotic resistance is that which evolves in populations of susceptible commensal and pathogenic bacteria colonizing and infecting hosts that are under antibiotic treatment or prophylaxis. Acquired antibiotic resistance can result in treatment failure and contribute to transmissible or primary antibiotic resistance. The research proposed in this application will be devoted to understanding, in a quantitative and predictive way, the genetic, bacterial, host factors and population dynamic processes responsible for the evolution of acquired resistance in populations of bacteria infecting uncompromised mammals treated with single and multiple antibiotics. Towards this end, we will develop and analyze the properties of mathematical and computer simulation models of the within-host population dynamics of antibiotic treatment and the evolution of resistance and perform in vitro and in vivo (laboratory mouse) experiments with a capsulated E. coli (O18:K1 :H7). In these experiments, we will estimate the parameters of these models, evaluate the reality of the assumptions behind their construction and test the validity of the predictions made from the analysis of their properties. The goals of this investigation are to; (1) Elucidate the conditions (dosage levels and treatment regimes) under which selection will favor the evolution of resistance in uncompromised mammals infected with antibiotic susceptible bacteria and treated with single antibiotics, multiple antibiotics and antibiotics for which clinical resistance requires multiple mutations. (2) Evaluate the contribution of post antibiotic effects (delays in the resumption of normal growth of antibiotic exposed bacteria after antibiotics are no longer at inhibitory concentrations) to the evolution of acquired resistance in populations of bacteria infecting antibiotic treated mammals. (3) Determine the contribution of elevated mutation rates to evolution acquired antibiotic resistance and the conditions under which antibiotic-mediated selection will result in the evolution of genes that augment mutation rates, mutator genes. The proposed research directed at these goals is in part, motivated by an academic interest in the mechanisms of adaptive evolution in bacteria. This research is also motivated by its direct utility to the health sciences and, in particular, to facilitate the design and evaluation of clinically effective antibiotic treatment protocols that minimize the likelihood of acquired antibiotic resistance evolving in the target population of bacteria.

**Grant:** 2R01AI040915-06A1  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** RAULSTON, JANE E PHD  
**Title:** CHLAMYDIA TRACHOMATIS ENVELOPE COMPONENTS AND VIRULENCE  
**Institution:** EAST TENNESSEE STATE UNIVERSITY JOHNSON CITY, TN  
**Project Period:** 1998/04/01-2009/02/28

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

**Grant:** 2R01AI042015-06A1  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** VIMR, ERIC R PHD  
**Title:** Regulation of the (poly) sialic virulence factor  
**Institution:** UNIVERSITY OF ILLINOIS URBANA- CHAMPAIGN, IL  
CHAMPAIGN  
**Project Period:** 1998/07/01-2008/12/31

DESCRIPTION (provided by applicant): Bacterial sepsis and meningitis are the leading causes of mortality and hospitalization by infectious agents in the United States. Sepsis alone accounts for >700,000 life-threatening infections each year and over 200,000 deaths, with economic losses greatly exceeding one billion annually. Extraintestinal pathogenic *Escherichia coli* (ExPEC) are the predominant agents of these infections, with one serotype, *E. coli* O18:K1:H7, emerging as the leading cause of gram-negative neonatal meningitis and the most prevalent isolate from uncomplicated cystitis in women, thus making it a useful model of ExPEC infections in general. How ExPEC breach the host's mucosa and reach the systemic concentration (bacteremia) necessary for triggering sepsis and meningitis is not understood, although cell-surface modification with sialic acid is an important determinant. In this continuation application, we propose to complete our studies to determine how the sialic and polysialic acid virulence factors are synthesized, and then to identify other gene products required for systemic disease using a functional genomic approach. Specific Aim 1 will complete the analysis of sialic acid biosynthesis and the regulation of sialate metabolism. Preliminary data indicate that the synthesis of N-acetylmannosamine (ManNAc), the first committed step in sialate biosynthesis, does not involve ManNAc 6-phosphate. X-ray crystallography and NMR spectroscopy will complement the objectives of this aim. In Specific Aim 2, the structural basis for PSA biosynthesis will be determined by analyzing chimeric polysialyltransferases (polySTs) and using site-directed mutagenesis to understand catalytic mechanism. Our preliminary data indicate that these important enzymes are structurally and functionally distinct from mammalian polySTs, suggesting wide therapeutic potential for specific polyST inhibitors. Specific Aim 3 will apply signature-tagged mutagenesis to identify disease traits that are either distinct from PSA or function in concert with this capsule to define ExPEC virulence. Our objective in this aim is to establish the minimal systemic ExPEC "pathotype". The proposed studies have direct application to the development of new therapeutic approaches by identifying novel targets for non-antibiotic drug or vaccine design. New approaches are urgently needed to prevent or treat ExPEC infections in the rapidly aging US population, the very young and the increasingly large number of immunocompromised patients.

**Grant:** 2R01AI042053-06A1  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** JERSE, ANN E PHD  
**Title:** Gonococcal Infection and Gene Expression in Female Mice  
**Institution:** HENRY M. JACKSON FDN FOR THE ADV ROCKVILLE, MD  
MIL/MED  
**Project Period:** 1999/02/01-2009/05/31

DESCRIPTION (provided by applicant): The capacity of *N. gonorrhoeae* to evade innate defenses in the female genital tract is hypothesized to be multifactorial and complex. Antioxidant factors may protect gonococci from killing by reactive oxygen species produced by phagocytes. Sialyltransferase (Lst) and other factors promote evasion of complement-mediated defenses. Evidence that these factors protect gonococci against evasion of innate host defenses is based primarily on in vitro assays. With the support of the first award, we developed the first reproducible small animal model of gonococcal genital tract infection. This model provides us with a valuable and unique research tool to test gonococcal interactions with host innate defenses. To satisfy the need for in vivo studies on factors hypothesized to contribute to evasion of PMN and complement-mediated killing, here we will i.) measure the relative contribution of the known antioxidant defenses of *N. gonorrhoeae* (catalase, cytochrome C peroxidase, manganese uptake, methionine sulfoxide reductase) in protection from killing by human PMNs and in survival during experimental murine genital tract infection. We will construct single and double mutants in genes hypothesized to directly defend against oxidative stress (*kat*, *ccp*, *mntC*, *msrA*), and test their capacity to survive opsonophagocytic killing by human and murine PMNs, and to infect normal mice and NADPH oxidase-deficient mice; ii.) define the role of gonococcal sialyltransferase in conferring resistance to opsonophagocytic killing by murine PMNs and in enhancing survival of *N. gonorrhoeae* in the murine lower genital tract We will determine if Lst-deficient gonococci are more sensitive to PMN killing due to increased uptake or the induction of a stronger respiratory burst. We will utilize C3 and C4-deficient mice and NADPH-deficient mice to test predictions made from PMN killing assays, iii.) Determine the basis for the observed increased infectivity of anaerobically grown *N. gonorrhoeae* for estradiol-treated mice and for increased resistance to the bactericidal activity of normal human serum. We will test mutants in genes that may confer increased survival in vivo as identified by DNA microarray technology to see if they are responsible for anaerobically-induced increased infectivity. We will assess the role of anaerobically induced nitrite reductase (*AniA*) in conferring an advantage in vivo and increased resistance to serum by testing the infectivity of an *aniA* mutant in mice, and by utilizing *AniA*-specific antiserum to block interactions between anaerobically grown gonococci and complement.

**Grant:** 2R01AI042154-06A2  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** RODERICK, STEVEN L PHD  
**Title:** Structure and Function of Hexapeptide Acyltransferases  
**Institution:** YESHIVA UNIVERSITY BRONX, NY  
**Project Period:** 1997/12/01-2008/12/31

DESCRIPTION (provided by applicant): Hexapeptide acyltransferases are characterized by tandem repeated copies of a six residue 'hexapeptide repeat' periodicity theme that encode folding of a unique left-handed parallel beta-helix structural domain. These enzymes play important roles in detoxification, cell wall formation and amino acid biosynthesis, but are absent in humans. Three bacterial hexapeptide acetyltransferases play well understood biological roles, but have not been mechanistically characterized or developed as antimicrobial drug targets. The gram-positive streptogramin acetyltransferase (VatD) inactivates the group A component of streptogramin mixtures currently used as last resort antibiotics in human medicine. High throughput screening and structure determination of enzyme-ligand complexes will be used to characterize the interaction of inhibitors with Vat(D). These compounds, or their synthetic variants, may find use as antibiotics that restore the susceptibility of gram positive pathogens to existing streptogramin mixtures. The bifunctional uridyltransferase (GlmU) is responsible for the biosynthesis of UDP-GlcNAc, an essential building block for both the peptidoglycan and lipopolysaccharide components of bacterial cell walls. Its acetyltransferase active site is unique but has not been mechanistically characterized. Its structure and mechanism of action will be defined in order to develop the potential of GlmU as an attractive mechanism-based antibacterial target. Serine transacetylase (STA) O-acetylates serine in the cysteine biosynthetic pathway of bacteria and is the key regulatory enzyme of cysteine biosynthesis and sulfate assimilation. The structure and reaction mechanism of STA will be studied as a resolved enzyme and as a component of the binary cysteine synthase complex. STA is inhibited by cysteine, but must also utilize serine. The means by which STA recognizes these isosteric amino acids as inhibitor and substrate, respectively, may point to features of antimicrobial agents that duplicate this natural high-affinity mode of inhibition. The successful completion of these aims will serve to advance basic functional understanding of the hexapeptide acetyltransferase superfamily of enzymes and will develop their application as screening-based or mechanism-based targets for antibacterial drug design.

**Grant:** 2R01AI042236-06  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** GROISMAN, EDUARDO A PHD MOLECULAR GENETICS  
**Title:** Molecular and Structural Bases of Polymyxin Resistance  
**Institution:** WASHINGTON UNIVERSITY ST. LOUIS, MO  
**Project Period:** 1998/02/01-2008/12/31

**DESCRIPTION** (provided by applicant): The unremitting isolation of bacterial pathogens exhibiting resistance to multiple antibiotics demands the development of novel therapeutic strategies. This entails not only identifying resistance determinants but also understanding the environmental cues that regulate expression of such determinants. The *Salmonella* PmrA/PmrB two-component system is required for resistance to the peptide antibiotic polymyxin B and to several antimicrobial proteins from human polymorphonuclear leukocytes. The regulatory protein PmrA governs expression of proteins mediating modifications of the lipopolysaccharide (LPS) with 4-aminoarabinose, which confers polymyxin resistance, and with phosphoethanolamine, the significance of which has remained unknown. We have established that: (1) the PmrB protein is a sensor that responds to extracellular levels of Fe<sup>3+</sup> by activating the PmrA protein, (2) low Mg<sup>2+</sup> can also activate the PmrA protein in a process mediated by the PmrD protein, and (3) mutants defective in the *pmrA* or *pmrB* genes are hypersensitive to killing by Fe<sup>3+</sup>. This proposal describes experiments aimed at understanding the cascade of events by which multiple environmental cues activate the PmrA/PmrB two-component system to promote resistance to different compounds. We will examine the molecular mechanism by which low pH activates the PmrA protein; investigate how the PmrD protein can activate the PmrA protein at a posttranscriptional level; identify the PmrA-regulated determinants mediating resistance to polymyxin and to Fe<sup>3+</sup>; and define the physiological role of PmrA-controlled phosphoethanolamine modification of the LPS. An accomplishment of these goals will uncover the molecular bases for bacterial signal transduction and antimicrobial peptide resistance. Moreover, as a variety of peptides and proteins are currently being developed as novel antimicrobial agents, the proposed experiments may help in our understanding of how these compounds exert their microbicidal properties.

**Grant:** 2R01AI042287-05A2  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** SOLOSKI, MARK J  
**Title:** T CELL RESPONSES TO INTRACELLULAR BACTERIAL PATHOGENS  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 1998/08/01-2009/06/30

DESCRIPTION (provided by applicant): In the last several years, significant progress has been made in understanding the relevance of the T cell immune response to the clearance of the Gram-negative pathogen *Salmonella typhimurium*, a category B pathogen. We have established that CD8+ T cells are essential to the protective immune response against infection with *S. typhimurium*, and these studies led to the identification of peptide epitopes recognized by bacteria-specific CD8 effector T cells. In this revised competitive renewal, we build on these initial observations and utilize a natural infection model. This model has allowed us to identify novel intestinal intraepithelial lymphocytes (iIELs) that expand following infection. These cells may represent a early element of the host mucosal response to infection. In the next five years, we will focus our efforts on the following Aims: Aim 1. What are the characteristics of the novel CD8+ alpha/beta expressing Intraepithelial Lymphocytes (iIELs) that are induced in the small intestine after oral infection with *S. typhimurium*? Specifically, we will determine their TCR usage, kinetics of appearance and define the recognition properties of these T cell subsets. Aim 2. What role does this novel CD8+/expressing iIELs play in the host immune response to *S. typhimurium*? Aim 3. Do bacteria, that display defined differences in cellular tropisms (e.g., dendritic cells vs. macrophages vs. epithelial cells), vary in their ability to stimulate host CD8+ T cell-mediated immune responses? The studies contained in this proposal are designed to address the immune elements that contribute to the clearance of infection and the generation of protective immunity to the Gram-negative pathogen *Salmonella typhimurium*. We hope to apply this information to the design of vaccine strategies that will evoke potent protective immunity as identify targets for immunotherapeutic strategies. We argue that such studies may aid in the design of novel strategies that can be used to stimulate immunity to an organism considered a bioterrorist threat. Also, such studies may contribute to understanding the etiological link between infection with gram-negative! pathogens in the development of autoimmune disease. Given that many of the cellular receptors in the mouse model have human counterparts, we argue that this murine model will yield valuable information that may be applied to the human setting.

**Grant:** 2R01AI042308-06  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** DERBYSHIRE, KEITH M PHD  
**Title:** Conjugation and recombination in mycobacteria  
**Institution:** WADSWORTH CENTER RENSSELAER, NY  
**Project Period:** 1999/02/01-2009/02/28

**DESCRIPTION** (provided by applicant): Mycobacterium tuberculosis accounts for over 2 million deaths per year. Furthermore, the global burden of tuberculosis has been compounded by its deadly association with the AIDS virus and by the emergence of multi-drug resistant strains, which have increased the demand for new treatments to stem the tuberculosis/AIDS epidemic. The design of new drugs and vaccines requires an understanding of the biology of mycobacteria and the development of genetic tools to manipulate their genomes in order to determine the molecular basis of pathogenesis and drug resistance. Although both transformation and transduction have had important roles in the development of mycobacterial genetics, little is known about conjugal DNA transfer. Conjugation offers an important alternative for transferring DNA between mycobacteria and, in particular, as a gene delivery system for moving markers between strains and generating targeted mutations. During the last grant-period, we described a DNA transfer process in Mycobacterium smegmatis that is different from any conjugation system described to-date. This proposal is designed to characterize the M. smegmatis conjugation system by defining and identifying both cis-acting DNA sequences and trans-acting proteins that mediate DNA transfer. Such analyses will provide important mechanistic information about the process of DNA transfer and allow differences between donor and recipient cells to be determined. Moreover, by modifying transferable plasmids, a new allele-exchange system will be established, enabling the capture of segments of chromosomal DNA and the generation of targeted mutations by transfer-mediated recombination. The application of this system to the slow-growing mycobacterial pathogens will be a valuable new addition to current molecular approaches. In addition, plasmids have been isolated from Mycobacterium avium that encode DNA relaxases related to those required for classical conjugal transfer in gram-negative bacteria. The ability of these plasmids to transfer among slow-growing mycobacteria will be investigated to understand the role of conjugation in lateral transfer among mycobacterial populations and its possible role in the spread of drug resistance. The aims are: 1. To characterize cis-acting sequences required for DNA transfer and to develop transfer as a molecular genetic tool for the study of mycobacteria. 2. To identify and characterize trans-acting transfer functions in both donor and recipient cells. 3. To examine transfer of chromosomal and plasmid DNA among fast- and slow-growing mycobacteria.



**Grant:** 2R01AI042797-07A1  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** SCHNEEWIND, OLAF MD  
**Title:** Targeting of Yop Proteins by *Yersinia enterocolitica*  
**Institution:** UNIVERSITY OF CHICAGO CHICAGO, IL  
**Project Period:** 1998/04/01-2008/11/30

**DESCRIPTION** (provided by applicant): Many Gram-negative pathogens use the type III pathway to transport protein toxins across the bacterial cell envelope. Pathogenic *Yersinia* spp., *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*, secrete 14 proteins (Yops, *Yersinia* outer proteins) via the type III pathway. During infection, substrate recognition and transport are regulated in a manner that allows the *Yersinia* type III pathway to direct Yops to the extracellular medium or into the cytosol of host cells. Together the type III mechanisms are essential to provide for bacterial escape from the host's innate immune response and to allow for microbial multiplication in human lymphoid tissues. Although many tissue culture cells serve as targets for type III injection, only some, but certainly not all cells of an infected host are injected by *Yersinia*. *Yersinia* lacking the surface adhesins YadA and invasin (InvA) fail to bind and inject tissue culture cells, suggesting that binding of bacterial ligands to surface receptors of host cells is a prerequisite for the type III injection of Yop proteins. During infection, *Yersinia* perceive host-specific environmental cues that are transduced to relieve repression of the type III pathway. Although several regulatory mechanisms of gene expression and type III transport have emerged, the genes and signaling pathways that activate type III secretion are not yet established. Preliminary work suggests that the type III machinery may recognize its substrates via signals encoded in yop mRNA. These secretion signals, suggested to reside in the first seven to fifteen codons of yop mRNA, presumably act by promoting transcript specific codon recognition and/or binding of factors that couple ribosomal protein synthesis to type III secretion. Work proposed herein focuses on characterizing the elements, factors and mechanisms of substrate recognition and regulation of type III transport. Other work is aimed at characterizing the mechanism of *Yersinia* target cell recognition. Together these studies will enhance our understanding of the molecular mechanisms that control the establishment of *Yersinia* infection and may provide insight into future strategies for prevention or therapy of these bacterial diseases.

**Grant:** 2R01AI042858-07  
**Program Director:** ROTHERMEL, ANNETTE L.  
**Principal Investigator:** VELLA, ANTHONY T PHD  
**Title:** Proinflammatory Cytokines Block T Cell Death In Vivo  
**Institution:** UNIVERSITY OF CONNECTICUT SCH OF FARMINGTON, CT  
MED/DNT  
**Project Period:** 1999/07/01-2009/06/30

**DESCRIPTION** (provided by applicant): The natural adjuvant bacterial lipopolysaccharide (LPS) is a proinflammatory factor that conditions T cells to circumvent immunological tolerance. Although the mechanism of breaking T cell tolerance is unclear, many recent studies have linked innate and adaptive immunity suggesting that productive immune responses are a result of these interactions. LPS injection into mice prevents Ag-specific T cell deletion leading to the development of long-lived memory T cells that possess potent recall responses. We have proposed that circumvention of T cell deletion by LPS is linked to the activation of innate immunity with an important role for dendritic cells (DCs). Firstly, this may be through the action of cytokines and our recent studies suggest that the proinflammatory cytokine IL-18 may be central to preventing T cell deletion. A direct role for DCs, IL-18 and the MyD88 molecule, which links aspects of innate and adaptive immunity, will be systematically tested. Secondly, when exposed to proinflammatory conditions that break T cell tolerance, specific T cells are very difficult to remove from lymphoid tissue prior to clonal expansion. Specifically, Ag-reactive T cells are trapped and virtually undetectable by flow cytometry, even though they are detected in situ by immunohistochemistry. We propose experiments to test the idea that trapping is a stage of information exchange between components of the innate and adaptive immune systems. Thirdly, to determine how LPS-induced inflammation functions on a cellular level in vivo. Our hypothesis is that DCs receiving direct signals from LPS may behave differently towards Ag-specific T cells as opposed to DCs that receive indirect inflammatory signals. This idea will be tested in a transgenic mouse model that allows tracking of Ag-specific T cells concomitantly with specific-Ag presenters that can respond directly to LPS versus ones that cannot. Collectively, these studies will help decipher how LPS induces changes in the microenvironment to convert T cell tolerance to immunity.

**Grant:** 2R01AI043006-06A1  
**Program Director:** AULTMAN, KATHRYN S.  
**Principal Investigator:** AZAD, ABDU F  
**Title:** INTERSPECIFIC COMPETITION BETWEEN RICKETTSIAE IN TICKS  
**Institution:** UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD  
SCHOOL  
**Project Period:** 1998/03/01-2009/06/30

DESCRIPTION (provided by applicant): Our preliminary studies and those of others clearly demonstrate a dominance of a single species of spotted fever group rickettsiae in ixodid tick vectors, *Dermacentor variabilis* and *D. andersoni*. Extensive tick surveys in Montana, Ohio, California, and Maryland revealed the dominance of nonpathogenic rickettsiae in tick populations. The presence of nonpathogenic tick symbionts may account for the low infection rates of *Rickettsia rickettsii* in *D. andersoni* and *D. variabilis* thus resulting in patchy distribution of human cases of Rocky Mountain spotted fever in the US. This project was initiated to investigate the molecular consequences and epidemiological significance of multiple rickettsial infections in ticks. We have proposed that the interactive effects between obligate intracellular rickettsiae within individual ticks may be of sufficient relevance and magnitude to alter the vector competence of ticks. Thus, we have focused on rickettsial symbionts acquired transovarially by *D. variabilis* ticks, namely *Rickettsia montanensis* and *R. peacockii*. We have investigated the prevalence and relative efficiency of transovarial interference of *R. montanensis* within *D. variabilis* ticks as mediated by *R. rhipicephali*. We have also focused on the *D. variabilis* genes that are differentially expressed in response to rickettsial infection within tick ovarian tissues. We have constructed several tissue-specific cDNA libraries from infected and uninfected *D. variabilis*. For the renewal application, we are proposing to further refine and characterize molecular mechanisms underlying transovarial interference. Studies under first aim define the functional role of identified molecules that initiate the exclusion of secondary rickettsial infections of tick ovaries. The second aim will be focused on investigating the role of the selected tick molecules in the maintenance and transmission of rickettsiae.

**Grant:** 2R01AI043023-06A1  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** FRIEDMAN, DAVID I MD  
ANTHROPOLOGY:PHYSICAL  
**Title:** Role of phage in expression & transmission of STX genes  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 1998/12/01-2009/05/30

DESCRIPTION (provided by applicant): Enterohemorrhagic E. coli (EHEC) is a significant public health problem because of the serious nature of the sequelae that can result from the infection. EHEC infection can progress from bloody diarrhea to serious kidney disease, HUS, and even death. Shiga toxin (Stx), a major virulence factor in EHEC and the cause of HUS, is usually carried in the genome of bacterial viruses called phages. Because these phages grow either lytically, releasing progeny into the environment, or lysogenically, inserting their DNA into the bacterial genome (as prophages), they are important agents for horizontal transfer of stx genes. In a fraction of lysogens, the prophage switches to lytic growth through a process called induction. Our work has shown that induced prophages add to the infectious process through their roles in increasing gene copy number of stx and through action of their regulatory systems contributing to the expression and release of Stx. The experiments outlined in this proposal are designed to examine the process of induction of Stx phage 933W and its role in Stx production and how the intestinal environment contributes to this process. We have constructed a bacterial reporter system that provides a genetic mechanism for identifying those bacteria in which the prophage is induced causing expression of phage-encoded genes, including stx. We plan to make improvements in this system, transfer it to an EHEC strain, and use the constructed strain to study how the in vivo environment contributes prophage induction and, thus, to Stx expression and release. We have shown that phage 933W encodes a functional eukaryotic-like tyrosine kinase, Stk, that is expressed in EHEC strains that have 933W prophages. We propose experiments designed to determine if Stk contributes to the virulence of EHEC strains. These studies should provide new and important information on factors influencing the course of EHEC infections. Moreover, this information should also contribute in a general way to the understanding of how interactions of bacteria and their environment influence the activity of their prophages, many of which encode virulence factors.

**Grant:** 2R01AI043199-06A1  
**Program Director:** LAMBROS, CHRIS  
**Principal Investigator:** BERMUDEZ, LUIZ E  
**Title:** Genes Associated with *M. avium* Pathogenesis  
**Institution:** OREGON STATE UNIVERSITY CORVALLIS, OR  
**Project Period:** 1999/08/01-2009/05/31

DESCRIPTION (provided by applicant): Infections caused by *Mycobacterium avium* complex are common in AIDS patients, and in patients with chronic underlying lung disease, such as emphysema and bronchiectasy. Despite of the success of anti-HIV-1 therapy in reducing the cases of *M. avium* disease, recent published work has demonstrated that in many areas *M. avium* is an environmental bacteria for which increasing incidence of disease in humans is predictable by the increased percent of the population with predisposing conditions. The ability to survive in different environments in the host requires tight gene regulation. Because *M. avium* is resistant to most of the antibiotic markers and is difficult to transform by external genetic material, it was necessary to create specific systems to study pathogenesis. During the past 3.5 years of this grant we have developed or adapted molecular systems, such as a transposon mutagenesis, signature tagged mutagenesis (STM), and GFP promoter fusion library, that allowed us to begin dissecting the complex aspects of *M. avium* interaction with the host. Several virulence genes were identified that allow the bacterium to enter intestinal epithelial cells, and to survive in macrophages. In addition, the great majority of the genes identified *in vitro* are also associated with virulence *in vivo*. Our hypothesis is that *M. avium* has specific strategies to subvert the host cells. We propose to continue this work by: A- Investigating how *M. avium* virulence-related genes are involved in the mechanism of invasion of intestinal mucosal cells. Our studies thus far have determined that bacterial entry is associated with the activation of small GTPases Rho A, and Cdc 42, and phosphorylation of N-WASP. We now detail experiments to further dissect the host cells pathways needed for bacterial uptake, based on the hypothesis that two pathways are used to enter epithelial cells. B- Analyzing the function of virulence determinants that are involved in the ability to survive and replicate in macrophages. We have developed a screening for the isolation of transposon-mutagenized *M. avium* bacteria that does not inhibit phagosome-lysosome fusion, and fails to suppress vacuole acidification. An initial screen of 3000 mutants resulted in the identification of a number of virulent determinants, such as PPE genes, polyketide synthases, MmpL proteins, ABC transporters, and several genes of unknown function.

**Grant:** 2R01AI043268-06A1  
**Program Director:** NEAR, KAREN A.  
**Principal Investigator:** JACOBS, WILLIAM R PHD  
**Title:** INH-induced lysis of the HIV OI, M. tuberculosis  
**Institution:** YESHIVA UNIVERSITY BRONX, NY  
**Project Period:** 1998/07/01-2009/04/30

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

**Grant:** 2R01AI043316-05A1  
**Program Director:** PETERS, N KENT  
**Principal Investigator:** SHAFER, WILLIAM M  
**Title:** Development of Staphylococcal Human Peptides  
**Institution:** EMORY UNIVERSITY ATLANTA, GA  
**Project Period:** 1998/07/15-2009/02/28

DESCRIPTION (provided by applicant): The capacity of *Staphylococcus aureus* to express resistance to multiple antibiotics is a major global health problem. The recent emergence of strains with decreased susceptibility to vancomycin (VISA isolates) is particularly worrisome. New and effective antimicrobials against *S. aureus* are therefore needed. Recent attention has been paid to antimicrobial peptides (APs) as possible therapeutic agents in combating the problem of antibiotic-resistant strains of bacteria. Our research program and the studies of others have implicated human lysosomal cathepsin G (cat G) as a potent mediator of neutrophil killing of *S. aureus*. We have discovered an AP within the full-length cat G sequence that has potent in vitro activity against *S. aureus* (including VISA isolates and methicillin-resistant strains); this peptide comprises residues 117-136 of cat G and is termed CG 117-136. During the past funding period we successfully modified CG 117-136 to enhance its bactericidal activity. We also found that levels of staphylococcal susceptibility to CG 117-136 were linked to the major cold shock gene *cspA*. Loss of *cspA* expression due to Tn551 insertion mutagenesis or deletion resulted in decreased susceptibility of *S. aureus* to this peptide. These *cspA* mutations also resulted in decreased or increased expression of several proteins and loss of pigmentation production. Moreover, growth of *S. aureus* in the presence of a sublethal concentration of this AP impacted the expression of several genes. In this proposal we will use a combination of microbial genetic (mutant selection), biochemical (proteomic analysis) and molecular (microarray gene chip technology) techniques to extend these observations with the goal of defining how *S. aureus* responds to AP. We will first define the role of cold shock gene expression in determining levels of AP susceptibility and pigment production (Specific Aim 1). The mechanisms by which cold shock proteins (CSPs) regulate *S. aureus* genes will be determined (Specific Aim 2). As exposure to AP represents a stress situation for bacteria, we will examine the genomic response of *S. aureus* to CG 117-136 by determining the genes of *S. aureus* that are regulated at the level of transcription during exposure to APs (Specific Aim 3). The integration of the genetic, molecular and biochemical experiments proposed will advance our knowledge regarding the mechanism of AP-killing of *S. aureus* and how this pathogen may develop resistance to this activity. This information should help in the development of new effective peptide-based drugs that could be used in the future to combat staphylococcal diseases caused by antibiotic-resistant strains.

**Grant:** 2R01AI043356-06A1  
**Program Director:** PETERS, N KENT  
**Principal Investigator:** SMELTZER, MARK S PHD  
**Title:** sar-mediated regulation in *Staphylococcus aureus*  
**Institution:** UNIVERSITY OF ARKANSAS MED SCIS LTL LITTLE ROCK, AR  
ROCK  
**Project Period:** 1998/07/01-2008/11/30

**DESCRIPTION** (provided by applicant): We have demonstrated that clinical isolates of *Staphylococcus aureus* are more virulent than the prototype laboratory strain (RN6390) in our animal models of musculoskeletal infection. We have confirmed that the genomes of these isolates includes genes that are not present in RN6390, and we hypothesize that there is a subset of genes that defines musculoskeletal isolates from their less virulent counterparts. We will test this hypothesis in Aim 1. Additionally, we have established that regulatory circuits in clinical isolates are distinct by comparison to RN6390 both in terms of the wild-type strains themselves and their respective agr and sarA mutants. Our studies indicate that this is due to a regulatory imbalance in RN6390 that results in a phenotype dominated by agr. In contrast, the phenotype of clinical isolates is dominated by sarA. An important aspect of this is that clinical isolates have an enhanced capacity to form a biofilm. Consistent with this hypothesis is our demonstration that mutation of sarA results in a reduced capacity to form a biofilm. Importantly, that is true in all *S. aureus* strains other than RN6390. Based on this, we hypothesize that specific components of the sarA regulon are required for biofilm formation and/or adaptation to the sessile lifestyle. In Aim 2, we will test this hypothesis by correlating the transcriptional profile of the relevant sarA mutants with the profile observed in bacteria harvested from biofilms. This will be done using comprehensive microarrays representing the genomes of all seven of the sequenced strains of *S. aureus* as well as the virulent clinical isolates themselves. Finally, we have also confirmed that mutation of sarA also limits the ability of our clinical isolates to cause disease, and we hypothesize that the inability to form a biofilm may be responsible, at least in part, for this attenuation. In Aim 3, we will test this hypothesis by correlating the ability to form a biofilm with virulence in our infection models. To accomplish these goals, we will 1) determine whether the genome of *S. aureus* strains that cause musculoskeletal infection includes a subset of genes that contributes to their virulence and is absent in less virulent laboratory strains, 2) correlate transcriptional changes associated with mutation of sarA with the adaptive response required for persistence within a biofilm and 3) define the impact of genes identified in Aims 1 and 2 on biofilm formation and virulence by generating appropriate mutations in clinical isolates of *S. aureus* and evaluating the impact using in vivo models of biofilm formation and musculoskeletal disease. We believe these experiments will ultimately lead to the identification of novel therapeutic targets for the treatment and prevention of staphylococcal musculoskeletal infection.



**Grant:** 2R01AI043428-05  
**Program Director:** PETERS, N KENT  
**Principal Investigator:** ROSEN, BARRY P  
**Title:** Metal binding domains in metalloregulatory proteins  
**Institution:** WAYNE STATE UNIVERSITY DETROIT, MI  
**Project Period:** 2000/08/01-2009/06/30

DESCRIPTION (provided by applicant): Exposure to drugs and toxic metals results in the acquisition of resistance mechanisms. Bacterial resistances are nearly all transcriptionally regulated. The overall goal of this study is to gain insights into the evolution and organization of novel metal binding motifs in the regulatory proteins that control expression of bacterial resistances. The clinically isolated resistance plasmids R773 and p1258 carry the arsenical resistance (ars) and cadmium resistance (cad) operons that encode ATP-coupled extrusion pumps for As(III)/Sb(III) and Pb(II)/Cd(II)/Zn(II), respectively. The ArsR and CadC repressors are two small homologous metal binding proteins responsible for metalloregulation of gene expression of the ars and cad operons, respectively. In plasmid-encoded ars operons there is a second unrelated As(III)/Sb(III)-responsive repressor, ArsD. Recent evidence indicates that ArsD serves as a metallochaperone for the As(III)- translocating ArsAB pump. Specific Aim 1. Structure and function of the S. aureus plasmid p1258 CadC: Two distinct types of metal sites, one within the DNA binding site and the other at the dimer interface, are observed in the crystal structure of CadC. The properties and function of each site will be explored by using a combination of molecular genetic, biochemical, biophysical and structural approaches. Specific Aim 2. Structure and function of ArsR As(III)-responsive repressors: Two aspects of ArsR structure and function will be analyzed. First, conformational change induced by As(III) binding will be probed. Second, the evolution of As(III) binding sites will be explored. Specific Aim 3. Roles of ArsD as a metalloregulator and a metallochaperone: The properties of ArsD that allow it to function as a repressor of the ars o/p will be explored, as will its role as a metallochaperone for intracellular transport of As(III) to the ArsAB As(III) extrusion pump. The ars repressors and homologues provide valuable models for the study of the regulation of drug and metal resistances: we have the ability to combine classical bacterial genetics and modern molecular biology with biochemical, biophysical and structural approaches.

**Grant:** 2R01AI043456-05A1  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** VAN VOORHIS, WESLEY C MD  
**Title:** Surface Antigens of *Treponema pallidum*  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 1999/09/28-2009/03/31

DESCRIPTION (provided by applicant): The studies in this proposal will concentrate on the protein Tp92 from *Treponema pallidum*. This molecule was discovered during our initial granting period, and due to the predicted central role this molecule appears to play in *T. pallidum* pathogenesis it will be our primary research focus for this grant submission. Factors favoring the study of Tp92 include the observations that: 1) Tp92 homologs are widely distributed throughout gram-negative bacterial species; 2) Tp92 homologs are surface-exposed in *Haemophilus influenzae* and *Pasteurella multocida*, and elicit protection in an animal model of infection for each of these pathogens; 3) Tp92 knockout mutants have been non-viable in several bacterial species, suggesting this molecule is required for bacterial survival; 4) the *T. pallidum* Tp92 is a target of opsonic antibody; 5) immunization with Tp92 is partially protective for challenge against *T. pallidum*; 6) Tp92 does not appear to undergo antigenic variation in *T. pallidum*; and 7) preliminary evidence suggests the *T. pallidum* Tp92 facilitates cell binding through host cell integrin molecules. The long-term objective of these studies is to further our current knowledge of *T. pallidum* pathogenesis by providing an in depth and detailed study of one of the key molecules involved in pathogenesis of this bacterium. The following four specific aims are proposed: Aim 1. Investigate the cell-binding function of the Tp92 molecule. Aim 2. Determine the potential of the putative chaperone Tp0327 to interact with Tp92 and other *T. pallidum* outer membrane proteins. Aim 3. Express Tp92 in a heterologous system to determine localization, interaction with Tp0327, and for epitope mapping. Aim 4. Map key determinants of Tp92 for protection and for the immune response. It is expected that these studies will provide insights into the function of the Tp92 protein in binding cells via integrins, which is likely to be a pathogenic mechanism of *T. pallidum*. It is also expected that these studies will define the interaction of Tp92 with a putative chaperone and this interaction is likely to be necessary for proper insertion of Tp92 in the outer membrane. Finally, these studies will help define the protective immune response that results after immunization with Tp92 as well as the immune response to Tp92 that occurs during infection.

**Grant:** 2R01AI043521-07A1  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** GUNN, JOHN S PHD  
**Title:** Salmonella Antimicrobial Peptide Resistance  
**Institution:** OHIO STATE UNIVERSITY COLUMBUS, OH  
**Project Period:** 1998/07/15-2008/12/31

DESCRIPTION (provided by applicant): Salmonellae are facultative intracellular pathogens that cause disease in humans and animals, including enteric (typhoid) fever and gastroenteritis. Typhoidal and non-typhoidal salmonellosis continues to cause significant morbidity and mortality worldwide. The overall objectives of this work are to better understand the induction of pathogenic bacterial gene expression in response to eukaryotic cell environments, as well as how bacteria utilize regulatory networks induced within these environments to avoid host innate immune killing. Antimicrobial peptides (AP), found at mucosal surfaces and within phagocytes, are a key weapon in the host innate immune arsenal. Two-component regulatory systems enable bacteria to sense their external environment and to mount an adaptive response by altering gene expression. Two such systems in Salmonella that are induced within the host during infection (PhoP-PhoQ; PmrA-PmrB) interact to remodel the outer membrane, including the lipopolysaccharide (LPS), which is the primary surface molecule that interacts with AP. We have recently identified a third member of this two-component cascade, UblA-UblB. PmrA-PmrB mediated modifications render the LPS less anionic, which leads to a reduced sensitivity to cationic AP, and these modifications have been shown to be necessary for oral virulence in mice. Further study of the in vivo induced PmrA-PmrB system and its role in LPS modification and virulence is necessary to better understand Salmonella pathogenesis and resistance to host innate immune killing. The aims of this grant are: (1) Characterization of novel PmrA-PmrB-regulated genes, (2) The role of PmrA-PmrB-mediated LPS modification in Salmonella virulence, and (3) The interaction of the UblA-UblB and PmrA-PmrB two-component regulatory systems. Understanding these regulated mechanisms by which salmonellae survive within the animal host could lead to novel therapeutic, preventative and diagnostic strategies, and are likely to be applicable to the studies of other bacterial pathogens of humans.

**Grant:** 2R01AI043643-04A2  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** EATON, KATHRYN A DVM VETERINARY MEDICINE  
**Title:** Host and Bacterial Factors in Disease due to H. pylori  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 1998/05/01-2008/04/30

DESCRIPTION (provided by applicant): H. pylori has been called the most common infectious disease of humans in the world today. Worldwide, between 50-100% of people are infected with H. pylori, but only a minority of those develop clinical signs of disease. Almost 2 decades of research have resulted in a general consensus that both host and bacterial factors contribute to disease, but the specific bacterial factors involved and the mechanisms whereby they promote colonization and induce severe manifestations of disease are not well understood. The overall goal of this project is to investigate these mechanisms. In the first funding interval we developed a mouse model of severe disease in which the contributions of host and bacterial factors to severe manifestations of disease can be evaluated. We utilized this model to determine the T cell subsets and cytokines that contribute to disease, we identified one bacterial factor, lipopolysaccharide O-antigen, that induces a deleterious host response and thus contributes to the outcome of disease, and we identified an H. pylori promoter, cagI5, that is upregulated in vivo and likely represents a new virulence factor. In this renewal, we will investigate the roles of O-antigen and cagI 5 in H. pylori pathogenesis, and use a newly-developed promoter trap to identify H. pylori genes that are upregulated in vivo. The 3 specific aims are: Specific aim 1: To test the hypothesis that cagI5 has a role in survival of H. pylori in vivo, and to determine the role of the cagI5 gene product in colonization and disease. Specific aim 2: To use a ureB reporter construct for promoter trapping, to identify novel colonization factors that are induced by growth in vivo, and to test the hypothesis that upregulated genes are essential for or facilitate colonization by and/or gastritis due to H. pylori. Specific aim 3: To test the hypothesis that the polysaccharide moiety of H. pylori lipopolysaccharide induces gastritis by receptor-mediated activation of antigen presenting cells. Successful completion of these aims will lead to improved understanding of the pathogenesis of H. pylori associated disease and provide a foundation for development of novel therapies.

**Grant:** 2R01AI043987-06  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** CIANCIOOTTO, NICHOLAS P PHD  
**Title:** Type II Secretion and Legionella pneumophila Infection  
**Institution:** NORTHWESTERN UNIVERSITY EVANSTON, IL  
**Project Period:** 1998/12/01-2008/11/30

DESCRIPTION (provided by applicant): Legionella pneumophila (Lp) is the agent of Legionnaires' disease pneumonia. In its aquatic habitat, Lp survives as an intracellular parasite of protozoans, and after inoculation into the lung, it flourishes within alveolar macrophages. Previously, we discovered an Lp gene (pilD) whose product is homologous with PilD, which, in other Gram-negatives, mediates pilus biogenesis and type II protein secretion. Indeed, an Lp pilD mutant lacked pili. More importantly, the pilD mutant, but not a pilin mutant, was defective for infection of amoebae and macrophages, suggesting that Lp has a type II secretion system that promotes infection. During this last grant period, we confirmed Lp has a type II system (lsp) that mediates secretion of many proteins, including novel enzymes, and is critical for infection of both protozoa and human cells. Some of our other data suggested that the Lp peptidyl-prolyl isomerase Mip and SurA have a role in the secretion process. Presently, Lp is the only known system for studying type II secretion in an intracellular pathogen. In the last grant period, we also demonstrated that lsp mutants are greatly impaired for survival in the lungs of A/J mice, with the severity of their defect indicating that Lp type II secretion is a key virulence determinant that may be involved in more than just macrophage infection. Finally, we made the novel observation that lsp is required for growth at 12-25 degrees C. Thus, Lp type II secretion is uniquely critical for intracellular infection, virulence, and low-temperature growth. In the current proposal, we aim to identify the type II exoproteins that are critical for intracellular infection, confirm the role of Mip and SurA in secretion, and determine how type II secretion promotes in vivo survival as well as low-temperature growth in water and amoebae. The results of these studies will i) increase our understanding of Lp physiology and pathogenesis, ii) provide new insight into bacterial protein secretion, intracellular infection, and low-temperature growth, and iii) have implications for other important human pathogens, including both other intracellular parasites and extracellular pathogens, such as Vibrio, Pseudomonas, and Burkholderia that survive at low-temperature and have type II systems.

**Grant:** 2R01AI044005-06  
**Program Director:** PERDUE, SAMUEL S.  
**Principal Investigator:** PALMER, GUY H  
**Title:** Antigenic variation in rickettsial transmission  
**Institution:** WASHINGTON STATE UNIVERSITY PULLMAN, WA  
**Project Period:** 1998/12/01-2008/11/30

DESCRIPTION (provided by applicant): The goals of the proposed research are to identify the epitope specificity and affinity required for immune control of antigenically-variant bacteria during acute and persistent infection and to determine whether this immune response is responsible for restricting pathogen genetic diversity in the mammalian reservoir host. This research addresses a fundamental gap in knowledge regarding control of bacterial pathogens in which the immune response is directed against both conserved and variable epitopes of outer membrane proteins. Tick-transmitted pathogens in the Family Anaplasmataceae (Order: Rickettsiales) cause acute febrile illness in animals and humans. During acute infection, cell-associated bacteremia reaches high, microscopically detectable levels and results in systemic disease. Importantly, ticks that feed on the mammalian host during this period of high-level bacteremia efficiently acquire the pathogen. Resolution of the acute high-level bacteremia requires CD4+ T lymphocytes and is associated with secretion of IFN-gamma and induction of neutralizing antibodies. This response does not completely clear the pathogen but the consequent persistent infection is controlled at low levels and the efficiency with which feeding ticks acquire the pathogen drops markedly. While studies have shown that persistence of *Anaplasma* spp. reflects emergence of organisms expressing structural and antigenic variants of the immunodominant outer membrane protein MSP2, how the immune response effectively controls the pathogen to low levels in face of this variation is unknown. In part 1 of the project, the epitope specificity and affinity of MSP2-specific CD4+ T cells associated with control of acute high-level bacteremia will be determined and whether induction of these responses prevents high-level bacteremia will be tested. In part 2, the epidemiological consequences of the gene conversion mechanism used to generate MSP2 variants and the effect of differential selection in the mammalian host versus tick vector will be examined. The generation of numerous complex MSP2 variants during infection of the mammalian host and the resulting immune responses are proposed to prevent tick-transmitted superinfection and thus restrict pathogen genotypic diversity. This hypothesis will be addressed using comprehensive identification of the variant population in the reservoir host and tick vector and testing whether immune responses against a broad array of variants prevents superinfection.

**Grant:** 2R01AI044072-06  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** ORME, IAN  
**Title:** Chronic Tuberculosis: Latent or Dynamic  
**Institution:** COLORADO STATE UNIVERSITY-FORT COLLINS, CO  
COLLINS  
**Project Period:** 1998/12/10-2009/03/31

**DESCRIPTION** (provided by applicant): This proposal from the Mycobacteria Research Laboratories, Colorado State University, is for competitive renewal of program AI-44072, "Chronic tuberculosis: latent or dynamic?" which in the previous funding period has made substantial progress in each of its Aims. In this new proposal, we wish to continue to try to define the immunological mechanisms that control and maintain the integrity of the chronic disease state, which we believe will ultimately provide a better understanding of the basis of latent tuberculosis in humans. In this proposal the first objective is to better define the role of IL-10, and to determine its potential role in reactivation disease in mouse strains prone to this event. The second Aim is to continue our studies on the role of CD8 T cells during the chronic disease state, given the accumulating evidence that they play an important role in maintaining the integrity of this process. A third Aim takes advantage of a new microarray laboratory recently established in the MRL. Using this facility we intend to try to provide a global picture of gene expression [concentrating initially on cytokines, chemokines, and their receptors] as the immune response in the lungs develops from containment, through the chronic disease state. Finally, in a fourth Aim, we propose to continue to develop new animal models that may provide new information about chronic/latent tuberculosis. The proposed work will take advantage of the advanced biosafety level III facilities at CSU, the broad expertise of various members of the Mycobacteria Research Laboratories, CSU, as well as a number of highly qualified consultants collaborators.

**Grant:** 2R01AI044167-06A1  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** ST GEME, JOSEPH W MD  
**Title:** Biology of H. influenzae Hia and Hsf Adhesins  
**Institution:** WASHINGTON UNIVERSITY ST LOUIS, MO  
**Project Period:** 1998/12/15-2009/04/30

DESCRIPTION (provided by applicant): Haemophilus influenzae is a common cause of localized respiratory tract disease, including otitis media, sinusitis, bronchitis, and pneumonia. Less commonly, this organism causes serious systemic disease, such as meningitis, septicemia, and endocarditis. The initial step in the pathogenesis of H. influenzae disease involves colonization of the upper respiratory mucosa. We have identified a non-pilus adhesin called Hia, which is present in nontypable (nonencapsulated) H. influenzae and promotes high-affinity attachment to human epithelium. In addition, we have identified a homolog of Hia called Hsf, which is present in nearly all encapsulated strains of H. influenzae and also mediates high-affinity adherence to epithelial cells. Both Hia and Hsf are members of the expanding family of auto-transporter proteins, characterized by an N-terminal signal peptide, an internal passenger domain, and a C-terminal trans-locator domain. In contrast to classic auto-transporters, Hia and Hsf have a translocator domain that is unusually short and forms a trimer. For both Hia and Hsf, the passenger domain has two distinct binding regions that interact with the same host cell receptor, although with differing affinities. We have crystallized a polypeptide containing the Hia primary binding domain and find a densely packed trimer with three-fold symmetry and three identical major grooves, acidic pockets, and globular domains. In Aim 1 of this proposal, we will define the surfaces of the Hia and Hsf binding domains involved in interaction with host cells. In addition, we will explore whether trimer formation is required for adhesive activity. In Aim 2, we will characterize the structure and mechanism of action of the Hia and Hsf translocator domain. In Aim 3, we will identify the Hia/Hsf host cell receptor and elucidate the host cell response to Hia and Hsf-mediated adherence. From a practical perspective, the results of these experiments may facilitate efforts to develop a vaccine protective against non-type b H. influenzae and suggest targets for novel antimicrobials with activity against a broad range of gram-negative bacteria. More generally, they may provide fundamental insights into host-pathogen relations, the autotransporter family of proteins, and protein secretion.



**Grant:** 2R01AI044198-06  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** TAN, MING MD  
**Title:** The Regulation of gene expression in Chlamydia  
**Institution:** UNIVERSITY OF CALIFORNIA IRVINE IRVINE, CA  
**Project Period:** 1999/08/01-2009/06/30

DESCRIPTION (provided by applicant): Chlamydia is the leading cause of sexually transmitted disease in the developed world, and preventable blindness in the developing world. Our long-term goal is to define the molecular mechanisms that regulate chlamydial gene expression so that we may intervene during the organism's intracellular developmental cycle. Our central hypothesis is that chlamydial gene expression is coordinately regulated at the transcriptional level by master regulatory molecules, such as activators and repressors, and by alternative forms of RNA polymerase. We propose three aims: 1) Investigate the function of a cis-acting DNA element that is important for Chlamydia-specific promoter activity. We will use biochemical and physical approaches to determine if a novel DNA element called the Spacer A/T region exerts its positive effect on transcription by binding an activator. 2) Define the role of HrcA, a transcription factor that regulates the expression of heat shock genes. We will determine how heat shock gene expression is regulated by the physical state of the HrcA repressor, higher temperature, DNA topology, and by the heat shock protein, GroEL. 3) Define the role of sigma28 RNA polymerase, an alternative RNA polymerase. We will use functional, bioinformatics and DNA microarray approaches to identify sigma28 promoters and sigma28-regulated genes.

**Grant:** 2R01AI044856-06

**Program Director:** SIZEMORE, CHRISTINE F.

**Principal Investigator:** SMITH, ISSAR PHD BIOLOGY NEC:BIOLOGY  
NEC-UNSPEC

**Title:** Molecular Determinants of M. tuberculosis virulence

**Institution:** PUBLIC HEALTH RESEARCH INSTITUTE NEWARK, NJ

**Project Period:** 1999/03/01-2009/02/28

DESCRIPTION (provided by applicant): Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), one of the oldest known human maladies, is still is one of the major causes of mortality, as two million people die each year from this disease. Despite the widespread use of an attenuated live vaccine and several antibiotics, there is more TB than ever before, requiring new vaccines, drugs and more specific and rapid diagnostics. The availability of the complete sequence of the Mtb genome and the use of new genetic and molecular methods has provided much new information concerning Mtb. The goal of the research described in this proposal, using this new information and methodology, is the identification of new targets in Mtb that will aid the development of these sorely needed anti-tubercular agents. The first period of research supported by grant AI-44856 was largely concerned with identifying Mtb genes and the proteins they encode that were potentially important in virulence. In the next grant period, we will investigate the roles in virulence played by genes that are induced in macrophages. We will also continue our studies on the sigma factors SigE and SigB and begin studies on SigL, concentrating on the genes that are transcribed by RNA polymerases containing these sigma factors, some of which, requiring sigma E, are necessary for Mtb virulence. The mechanism of regulation of sigma E and sigma L function by specific anti sigma factors will also be studied. We have shown that IdeR is the major regulator of Mtb iron flux. Since iron is essential for Mtb survival and virulence, we will identify and characterize iron/IdeR repressed genes and proteins they encode that form the iron acquisition machinery. IdeR is an indispensable protein, as its structural gene cannot ordinarily be inactivated, and we have shown that IdeR is also a positive regulator of iron storage genes. We plan to investigate the mechanism by which this protein activates gene expression, as this may help explain its essential nature.

**Grant:** 2R01AI045025-06  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** SHEN, HAO PHD  
**Title:** Cellular Immune Surveillance of Intracellular bacteria  
**Institution:** UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA  
**Project Period:** 1999/02/15-2009/01/31

DESCRIPTION (provided by applicant): The long-term objectives of our study are to understand 1) what bacterial antigens are recognized by the immune system and what factors influence the repertoire of antigenic targets in bacteria, 2) what immune mechanisms are protective and how host immune effectors counteract specific virulence factors to bring about protective immunity, and 3) how likely and by what mechanisms bacteria may escape immune surveillance. In this application, we will use *Listeria monocytogenes* (LM) as a model to: 1. Examine how regulation of gene expression affects the ability of a bacterial protein to induce immune responses and to serve as a protective target. The results of this study will help us define the complexity of the antigenic repertoire of bacteria and develop general guidelines for the selection of antigenic targets for vaccination. 2. Test a model that protective immunity against LM is determined by a race between CTL-mediated cytolysis and bacterial spread into neighboring cells. This model is suggested by our finding that CTL cytolysis functions to counteract LM's virulence strategy of direct cell-cell spread. We will test several predictions of this model and in doing so we hope to identify mechanisms of immune protection that correlate with bacterial virulence strategies. 3. Investigate the possibility that antagonist peptides may play a role in shaping the repertoire of T cell targets and in allowing bacterial escape of CTL surveillance. Our preliminary results have demonstrated the in vivo effect of TCR antagonism on T cell responses and protective immunity. We will study several aspects of agonist/antagonist interactions in vivo during infection. The results of these studies will help in our understanding of the complex interactions between bacteria and their hosts that determine the outcome of infection, and will have important implications for the design of effective vaccines and the selection of protective vaccine antigens.

**Grant:** 2R01AI045125-06  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** DIRITA, VICTOR J PHD MOLECULAR BIOLOGY,  
OTHER  
**Title:** Mechanism of Virulence Regulation by Membrane Activators  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 1999/03/15-2009/03/31

DESCRIPTION (provided by applicant): Virulence gene regulation in *Vibrio cholerae* requires the action of four unusual transcription regulatory proteins, pairs of membrane localized transcription activator/effector molecules called ToxR/ToxS and TcpP/TcpH. Working models for the mechanisms of action of these proteins hold that ToxR and TcpP collaborate to activate expression of a protein ToxT, which activates genes encoding virulence factors cholera toxin and toxin co-regulated pilus. By contrast, ToxR alone - independently of TcpP - can regulate expression of OmpU and OmpT, two outer membrane proteins. Interactions between the activators and effectors are predicted to take place in the periplasmic space, although in general the roles of the effectors are less well characterized. TcpH acts to block a proteolytic mechanism that targets periplasmic domain of the TcpP. Whether TcpH plays any other role in the function of TcpP will be assessed in this study. FoxS may serve to confer higher order structure on ToxR essential for its function. Specific hypotheses generated from these mechanistic models of membrane-localized activator function will be tested. Much of what we understand about these proteins has come from genetic and biochemical studies, and these will continue in the work proposed herein. With advances and imaging and cytological resources available for studying the prokaryotic cell, studies aimed at developing reagents and experimental approaches for studying membrane localization of transcription complexes are also proposed. Specific Aims I. Determine the mechanism of ompU and toxT activation by ToxR. II. Determine the role of DNA binding and RNA polymerase interaction by TcpP for toxT activation. III. Define the mechanisms and consequences of activator/effector periplasmic interactions. IV. Develop cytological methods for analyzing membrane localized activator function.

**Grant:** 2R01AI045148-07  
**Program Director:** JACOBS, GAIL G.  
**Principal Investigator:** DERETIC, VOJO P PHD MOLECULAR BIOLOGY,  
OTHER  
**Title:** Mechanism of *M. tuberculosis* Phagosome Maturation Arrest  
**Institution:** UNIVERSITY OF NEW MEXICO ALBUQUERQUE ALBUQUERQUE, NM  
**Project Period:** 1999/03/01-2009/06/30

**DESCRIPTION** (provided by applicant): The ability of *Mycobacterium tuberculosis* to infect an extraordinary number of people, combined with the widespread emergence of multidrug resistance and opportunistic infections in AIDS, has lead to its placement on the NIAID list of biodefense and emerging infectious agents. *M. tuberculosis* persistence in human populations rests upon its ability to infect and survive in macrophages. Intracellular *M. tuberculosis* inhibits phagosomal maturation and resides in a pathogen-friendly phagosome escaping lysosomal bactericidal mechanisms and efficient antigen presentation. The long-term objectives of this project are to characterize mycobacterial products responsible for the *M. tuberculosis* phagosomal maturation block and identify the host cell membrane trafficking processes that are targeted by the mycobacterial factors. These phenomena have been linked to mycobacterial interference with membrane trafficking and organelle biogenesis processes controlled by host cell Rab GTPases. We hypothesize that *M. tuberculosis* interferes with specific Rab-interacting partners, including a Rab effector, phosphatidylinositol 3-kinase, that prepares phagosomes for tethering and fusion with other organelles. An integral part of this hypothesis is that *M. tuberculosis* lipids, which mimic mammalian phosphatidylinositols, affect organellar fusion and phagosomal maturation by interfering with phosphatidylinositol 3-kinase-dependent processes in the host cell. The specific aims are: 1. Identify *M. tuberculosis* lipid and protein products affecting phagosome maturation and characterize their mode of action. 2. Delineate the role of host cell membrane trafficking regulators including Rab5 effectors in *M. tuberculosis* phagosome maturation arrest. 3. Characterize novel, cell biology-based processes that can counteract *M. tuberculosis* phagosome maturation arrest. This three-prong approach will improve our understanding of a marquee pathogenic determinant of *M. tuberculosis* and provide a foundation for new interventions potentially combating both active disease and latent infection.

**Grant:** 2R01AI045801-05A1  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** SCHWARTZ, IRA S.  
**Title:** B. burgdorferi Hematogenous Dissemination  
**Institution:** NEW YORK MEDICAL COLLEGE VALHALLA, NY  
**Project Period:** 1999/07/15-2009/06/30

DESCRIPTION (provided by applicant): Lyme disease, the most prevalent arthropod-borne disease in the United States, is caused by infection with the spirochete, *Borrelia burgdorferi*. Despite intensive study in recent years little is known regarding the pathogenesis of infection at the molecular level. We have determined the genetic diversity among clinical isolates of *B. burgdorferi* and shown that spirochete dissemination varies significantly in patients and mice infected with distinct genotypes. These results demonstrate that different genotypes of *B. burgdorferi* possess varying potential for dissemination in an infected host. We hypothesize that these differences are the result of variations in gene content and/or expression among different genotypes. We propose to employ functional genomics and proteomics to gain insight into possible differences in gene/protein expression between *B. burgdorferi* strains with differing capacities for hematogenous dissemination. The long-term objective of this project is elucidation of genes and/or proteins that mediate *B. burgdorferi* virulence. The following specific aims are proposed: 1) global gene expression of *B. burgdorferi* clinical isolates of differing genotype in varying environments will be monitored by gene array; 2) differences in protein expression among the various genotypes will be assessed by proteomics; 3) the roles of candidate virulence genes in pathogenesis of Lyme disease will be evaluated by monitoring expression for a number of the most promising candidate genes will be monitored in early Lyme disease patients and infected mice by real-time RT-PCR; 4) selected candidate virulence genes will then be targeted for insertional inactivation and complementation. The effects of such genetic manipulation should provide direct demonstration of the critical role such genes may play in spirochete pathogenesis. The combination of functional genomics, in vivo and genetic approaches proposed here should provide for the comprehensive assessment of the molecular basis of pathogenesis for *B. burgdorferi* isolates with varying potential for bloodstream dissemination and result in identification of determinants required for spirochete dissemination. This will provide new insights into the natural history of *B. burgdorferi* infection in humans, which, in turn, may have implications for prevention, treatment, and diagnosis of Lyme disease.

**Grant:** 2R01AI046097-06  
**Program Director:** JACOBS, GAIL G.  
**Principal Investigator:** ERNST, JOEL D  
**Title:** M. tuberculosis evasion of immune effector mechanisms  
**Institution:** NEW YORK UNIVERSITY SCHOOL OF MEDICINE NEW YORK, NY  
**Project Period:** 2000/06/01-2009/05/31

DESCRIPTION (provided by applicant): Mycobacterium tuberculosis (Mtb) causes more adult deaths worldwide than any other bacterium, and many strains are resistant to all first-line drugs. Although HIV infection is associated with a high rate of active tuberculosis (TB), Mtb can cause disease in people with normal immune systems, implying that Mtb can evade the immune response. We tested the hypothesis that Mtb inhibits effector mechanisms of the immune response, and found that Mtb inhibits macrophage (MO) transcriptional responses to IFN $\gamma$ . Studies during the present funding period revealed that Mtb utilizes 3 distinct mechanisms to inhibit MO responses to IFN $\gamma$ : an Mtb lipoprotein (LP) activates a TLR2-dependent mechanism for inhibition; Mtb peptidoglycan (PG) activates a TLR2- and MyD88-independent mechanism, and Mtb induces secretion of interleukin-6, which also inhibits MO responses to IFN $\gamma$ . We also found that Mtb-LP and Mtb-PG block IFN $\gamma$  priming of MO to kill Mtb, implying that inhibition of MO responses to IFN $\gamma$  contributes to persistence and progression of TB. The overall goal of the experiments proposed in this application is to define the mechanisms of inhibition of MO responses to IFN $\gamma$ , in order to develop the means to overcome the inhibition and enhance the efficacy of the immune response to Mtb. Specific Aim 1 is to define the structural features of Mtb PG that account for its 40-fold greater potency compared to E. coli PG for inhibition of MO responses to IFN $\gamma$ ; Specific Aim 2 is to determine the contributions of Mtb lipoproteins, peptidoglycan, and IL-6 to modulating gene expression and Mtb killing in MO, and Specific Aim 3 is to define the early, intermediate, and late steps in Mtb PG-induced inhibition of MO responses to IFN $\gamma$ , and compare them to the steps required for Mtb LP- and interleukin 6-initiated inhibition. The proposed experiments will provide high-resolution understanding of the mechanisms used by Mtb to block MO responses to IFN $\gamma$ , and will provide a basis for interventions to overcome the block and enhance immunity to Mtb.

**Grant:** 2R01AI046464-04A1  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** GRANOFF, DAN M MD  
**Title:** Novel vaccine approaches for N. meningitidis disease  
**Institution:** CHILDREN'S HOSPITAL & RES CTR AT OAKLAND, CA  
OAKLAND  
**Project Period:** 1999/12/01-2008/01/31

DESCRIPTION (provided by applicant): N. meningitidis is an important cause of meningitis and sepsis. Conventional approaches to develop a vaccine for prevention of disease caused by capsular group B strains, which account for 30-80% of all cases, have been largely unsuccessful. We propose to investigate two complementary vaccine approaches. First is a subunit vaccine based on our studies of three recombinant proteins, Neisserial protein A (NspA) and two antigens, designated NspD and E, which were discovered during the group B MC58 genome sequencing project. All three elicit bactericidal antibody responses against homologous and heterologous strains and, therefore could form the basis of monovalent or multivalent vaccines. Our second approach is based upon our discovery that sequential immunization with native vesicles prepared from three meningococcal strains, each differing by capsular group, PorA variable region sequence type, PorB serotype and LOS immunotype, overcomes strain-specific bactericidal antibody responses associated with conventional prime/boost immunization with vesicles vaccines, in part by enhancing the antibody responses to NspA. The anti-NspA antibody responses to sequential immunization with vesicle vaccines also are higher than those made to recombinant NspA. Our hypothesis is that repeated presentation of vesicle vaccines containing highly conserved antigens in the context of different, variable antigens such as PorA, which is usually immunodominant, enhances the response to the conserved antigen. In Aim 1, we will investigate improving the ability of recombinant NspA, D, or E to elicit protective antibody responses by reconstituting the recombinant proteins in liposomes or micelles to restore conformational epitopes. We also will prepare a panel of bactericidal mAbs to monitor expression of critical epitopes in the recombinant proteins, or improved vesicle vaccines (Aim 2). In Aim 2, we will construct specialized N. meningitidis strains to produce improved vesicle vaccines that are designed to both enhance immunogenicity by over-expressing NspA, D and E, and to minimize toxicity by genetically decreasing unwanted or potentially toxic antigens. In Aim 3, we will determine whether vaccines composed of more than one recombinant antigen, or vesicle vaccines combined with a recombinant antigen, can augment bactericidal antibody responses. The proposed studies will identify safe and more broadly protective vaccine strategies for prevention of N. meningitidis disease, including group B strains for which there is currently no vaccine available. The lessons learned also will be broadly applicable to advancing vaccines against other pathogens that target antigens identified by "genome mining."



**Grant:** 2R01AI046582-05

**Program Director:** SIZEMORE, CHRISTINE F.

**Principal Investigator:** KOLATTUKUDY, PAPPACHAN E PHD  
BIOCHEMISTRY:BIOCHEMISTR  
Y-UNSPEC

**Title:** Search for novel TB Drug Targets in Lipid Metabolism

**Institution:** UNIVERSITY OF CENTRAL FLORIDA ORLANDO, FL

**Project Period:** 2000/01/15-2008/12/31

DESCRIPTION (provided by applicant): Tuberculosis (TB) is a leading cause of preventable deaths accounting for over two million deaths per year. Natural spread of multiple drug resistant (MDR) TB is a major threat to public health. CDC has classified MDR strains of *M. tuberculosis* (MTB) in Class C, within the list of organisms with potential use in bioterrorism. Discovery of novel anti-mycobacterial drug targets is critically needed to combat these threats, especially the MDR TB. The MTB genome is unusually rich in genes for lipid metabolism. It is becoming increasingly clear that lipid metabolism plays critical roles in TB. The critical steps, that are uniquely required for infection and survival of the pathogens in a dormant state for decades before the pathogens develops active TB when the host becomes immunodeficient, can be ideal targets for novel anti-TB drugs. We postulate that tgs/wes genes (TG synthase/wax ester synthase genes) and lip genes are involved in virulence and in the survival of the pathogen under dormant conditions. We will test this hypothesis. 1) Elucidate the biochemical functions of tgs/wes gene products. a) Characterize the enzymatic activities of the tgs/wes gene products expressed in *E. coli*. b) Determine the biochemical consequences of disrupting each tgs/wes gene on lipid metabolism. 2) Determine the consequence of tgs/wes gene disruption on host-pathogen interactions, a) Determine whether any molecular changes relevant to induction of TG synthesis can be detected as MTB reaches the hypoxia-induced nonreplicating state in culture b) Determine whether mutants have altered ability to grow in macrophages and trigger cytokine production, c) Determine the virulence, persistence, and the ability of the tgs/wes mutants to go into dormancy in mice and cause infection when the host is immunocompromised. 3) Elucidate the biochemical functions of the lip genes a) Express and characterize the TG hydrolase and thioesterase activities of lip gene products expressed in *E. coli*. b) Disrupt each lip gene and determine the biochemical consequences. 4) Determine the effect of lip disruptants on host-pathogen interaction, a) Determine the ability of the lip gene disruptants to survive the hypoxia-induced nonreplicating state in culture, b) Determine the effects of lip gene disruption on growth in macrophages and cytokine production. c) Determine the effect of lip gene disruption on virulence, persistence and the ability to undergo dormancy and reactivation in immunocompromised host. 5) Elucidate the biosynthetic mechanisms involved in the production of dimycocerosylphthiocerol (DIM), a known virulence factor. Identification of the unique steps in lipid metabolism critical for the disease will allow a search for novel drugs directed at these targets.

**Grant:** 2R01AI048489-06  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** YOUNG, JOHN A T PHD  
**Title:** Anthrax Toxin Entry into Cells  
**Institution:** SALK INSTITUTE FOR BIOLOGICAL STUDIES LA JOLLA, CA  
**Project Period:** 2000/08/15-2009/06/30

DESCRIPTION (provided by applicant): *Bacillus anthracis*, the causative agent of anthrax, is one of the six CDC category A agents considered to be those which would cause the most adverse public health impact if used in a biological attack. Highly virulent forms of *B. anthracis* secrete two toxins, lethal toxin (LeTx) and edema toxin (EdTx), which are thought to be primarily responsible for the major symptoms and death associated with anthrax. Entry of both types of toxin into cells is mediated by the bacterial protective antigen (PA) subunit. In an effort to understand how these toxins enter cells we have identified two distinct cellular receptors for PA, Anthrax Toxin Receptor/Tumor Endothelial Marker-8 (ATR/TEM8) and Capillary Morphogenesis Protein-2 (CMG2). We have also developed soluble receptor decoys as candidate antitoxins for the treatment of anthrax. The research described in this proposal will build upon these landmark findings to further our understanding of anthrax toxin entry. The first aim will investigate the role played by the receptors in trafficking toxin to distinct sites in the cell that are associated with marked differences in toxin stability. The second aim will seek to develop a cell-free system that will allow the dissection of the molecular mechanism of anthrax toxin translocation. The third aim will investigate whether anthrax toxin, like other bacterial toxins, can be targeted to other types of cell surface receptors (surrogate receptors). This information will provide important insights into the critical parameters required for toxin entry. The fourth aim will seek to establish whether the DNI-class of anthrax antitoxins can block toxin entry that is mediated by surrogate receptors. Together, this body of work will contribute significantly to our understanding of the cell biology of anthrax toxin entry. This information in turn may reveal new targets for therapeutic intervention in the treatment of disease, and may pre-empt the actions of bioterrorists who seek to target PA to surrogate cell surface receptors in an effort to evade anti-PA antibody and soluble receptor decoy-based antitoxins.

**Grant:** 2R01AI048945-05  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** CONWAY, TYRRELL PHD  
**Title:** Growth and Colonization of the Intestine by E. coli  
**Institution:** UNIVERSITY OF OKLAHOMA NORMAN NORMAN, OK  
**Project Period:** 2000/06/15-2009/05/31

DESCRIPTION (provided by applicant): Escherichia coli is the predominant facultative anaerobe in the gastrointestinal tracts of mammals. Yet the essence of how E. coli colonizes its mammalian hosts is not understood. According to the nutrient-niche hypothesis, the numerous ecological niches in the intestine are defined by nutrient availability and species coexist by competing for one or a few limiting nutrients. This hypothesis suggests that commensal E. coli strains might act as a first line of defense against enteric E. coli pathogens, but it has not been tested in that light. In this competing renewal application, we propose to extend our investigation of the nutritional basis for intestinal colonization by testing the hypothesis that mucus-derived nutrients provide nutritional niches occupied by competing E. coli commensal and pathogenic strains. The first specific aim will test the hypothesis that different E. coli commensal and pathogenic strains preferentially use different nutrients for colonization of the mouse intestine. Work described in the progress report suggests that different E. coli strains use different nutrients for colonization. For strains representing the four phylogenetic groups of E. coli, the specific nutrient requirements during the initiation and maintenance stages of colonization will be determined by using strategies developed for the currently funded project, i.e., genes induced on DNA arrays under conditions designed to mimic the mouse intestine will be knocked out and the mutants tested in competition with their wildtype parent to determine their relative fitness for colonization. The second specific aim will test the hypothesis that preferential utilization of different nutrients and/or the ability to switch to alternative nutrition is the basis for co-colonization of E. coli strains representing each of the four phylogenetic groups. The corollary hypothesis will also be tested, that direct competition for the same nutrient leads to decreased fitness for colonization of one of the strains. These studies may help explain differences in human susceptibility to infection by E. coli pathogens.

**Grant:** 2R01AI049174-04A1  
**Program Director:** LAUGHON, BARBARA E.  
**Principal Investigator:** FAHEY, ROBERT C BS  
**Title:** MYCOTHIOI BIOSYNTHESIS AND METABOLISM AS TB DRUG TARGETS  
**Institution:** UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA  
**Project Period:** 2000/08/01-2007/05/31

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

**Grant:** 1R01AI049571-01A2  
**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:** 2004/05/01-2009/04/30

DESCRIPTION (provided by applicant): Chlamydia trachomatis (Ct) is the most common cause of bacterial sexually transmitted disease worldwide. In the majority of infected individuals they are asymptomatic. This poses a health risk for women, causing pelvic inflammation and tubal infertility, and for newborns from infected mothers. Our goal is to characterize the human T cell immune response to Ct in infected individuals with the goal of identifying immunogenic proteins and peptides that could be used for vaccine development. We will determine whether CD4 or CD8 T cell responses can be elicited against Ct proteins that enter the MHC class I or class II antigen processing pathway or are likely to enter those pathways. Specific peptide epitopes from these proteins and the HLA allotypes that present the peptides will be determined using a unique panel of B lymphoblastoid lines expressing single HLA antigens. Our hypothesis is that some regions of immunogenic proteins will contain epitope clusters for both CD4 and CD8 T cells as we found for the major outer membrane protein MOMP. The functional characteristics of the Ct-specific T cells will be determined using antibodies against cytokines, proteins involved in cytotoxicity, and cell surface molecules. Assays such as the Lysis spot assay will be used to determine CTL function. Ct-specific T cells in the blood of infected individuals will be enumerated with MHC class I tetramer and class II tetramers. We will determine if the CD8 T cells recognizing Ct antigens are functional CTL cells or potentially "exhausted" T cells lacking CTL activity, which has been found in chronic viral infections. The potential for immunogenic epitopes to cross-protect other Ct species such as C. pneumonia and C. psittaci will be determined by sequence comparisons. Characterizing human T cell responses to Ct is important for understanding immunity to Ct in humans and for future vaccine development.

**Grant:** 1R01AI050812-01A2  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** SCHURR, MICHAEL J PHD  
**Title:** Regulation of *Ps. aeruginosa* Virulence Factors by AlgR  
**Institution:** TULANE UNIVERSITY OF LOUISIANA NEW ORLEANS, LA  
**Project Period:** 2004/02/01-2009/01/31

DESCRIPTION (provided by applicant): *Pseudomonas aeruginosa* is ubiquitous, opportunistic pathogen that primarily infects immune-compromised individuals, including AIDS and transplant patients, severe burn patients, and those with cystic fibrosis (CF). In the context of CF, *P. aeruginosa* establishes a chronic condition whose morbidity and mortality results from lung damage. Due to the uncanny antibiotic resistance, CF patients infected with *Pseudomonas* often have chronic infections with limited therapeutic options. Therefore, for improved efficacy in treatment, a basic understanding of the pathogenic mechanisms utilized by this organism needs to be examined as possible therapeutic targets. While a majority of previous studies have focused on the initial stages of colonization and infection, we propose a new approach in searching for treatments. Mounting evidence indicates that microaerophilic metabolism and a biofilm mode of growth may be involved in *P. aeruginosa* pathogenesis; however, explanations for a mechanism have yet to be discussed. One virulence factor produced by *P. aeruginosa* under these growth conditions is HCN. Micromolar amounts of HCN inhibit the respiratory electron transport chain and several metalloenzymes (e.g., catalase, peroxidase, superoxide dismutase) of eukaryotic cells. We have discovered that AlgR, a regulator of the virulence factor, alginate, also activates HCN production in mucoid *P. aeruginosa*. Using the *Pseudomonas* Affymetrix GeneChip and S1 nuclease protection assays, we demonstrate that AlgR is controlling *hcnA*, encoding hydrogen cyanide synthase. Moreover, direct measurement of HCN production revealed that mucoid *P. aeruginosa* produce up to 2.5 mM of HCN in 4 h. Our preliminary data indicate two new roles for AlgR: i) AlgR controls HCN production and ii) AlgR is able to switch from a repressor in nonmucoid *P. aeruginosa* to an activator in mucoid bacteria on the *hcnA* promoter. Additionally, we demonstrate that AlgZ/FimS is playing a role in this process. The hypothesis to be tested is: AlgR activates HCN production in mucoid *P. aeruginosa*. We will test this hypothesis with four specific aims: i) we will determine the requirements for AlgR protein-DNA interaction within the *hcnA* promoter; ii) we will determine if phosphorylation is required for AlgR activation of *hcnA* expression in mucoid *P. aeruginosa*; iii) we will determine the amount of HCN production and *hcnA* expression within biofilms, and; iv) we will determine the effect of HCN production on lung epithelial and human neutrophil cells in vitro. Thus, at the end of our proposed studies, we hope to elucidate new possible therapeutic target as well as gaining a better understanding of *Pseudomonas* biology and pathogenesis.

**Grant:** 1R01AI051417-01A2  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** BAVOIL, PATRIK M PHD  
**Title:** POLYMORPHIC MEMBRANE PROTEINS OF CHLAMYDIA TRACHOMATIS  
**Institution:** UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD  
SCHOOL  
**Project Period:** 2004/05/15-2009/04/30

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

**Grant:** 1R01AI052147-01A2  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** CHAUSSEE, MICHAEL S BS  
**Title:** Regulation of Streptococcus pyogenes exoproteins  
**Institution:** UNIVERSITY OF SOUTH DAKOTA VERMILLION, SD  
**Project Period:** 2004/04/01-2008/03/31

DESCRIPTION (provided by applicant): Streptococcus pyogenes secretes several proteins to the extracellular environment that directly influence host-pathogen interactions and contribute to virulence. Many secreted proteins have been studied in detail; however, the functions of several others are unknown. The locus-designated rgg is required for the expression of streptococcal pyrogenic exotoxin B (SPE B), a secreted cysteine protease that contributes to virulence. Inactivation of rgg also altered the expression of additional secreted proteins. Results obtained from genetic and physiological analyses of the rgg mutant strain have led to the hypothesis that the composition of available catabolic substrates influences exoprotein expression in an Rgg-dependent manner. To test the hypothesis, the following aims are proposed: Specific Aim 1. Determine if the availability of nitrogen-containing catabolic substrates influences Rgg dependent expression of virulence-associated exoproteins. Quantitative RT-PCR will be used to identify changes in mf-1 and speB expression in response to the availability of catabolic substrates. Proteomics and metabolite analysis will be used to assess the influence of catabolic substrates on exoprotein expression. Specific Aim 2. Identify Rgg-regulated proteins. Differences in protein expression between wild-type strain NZ131 and an isogenic rgg mutant will be detected with two-dimensional gel electrophoresis and differentially expressed proteins identified with mass spectrometry. Specific Aim 3. Distinguish between Rgg-regulated proteins and changes in expression due to perturbations of other regulatory circuits. Changes in protein expression will be identified with proteomics following induction of rgg expression by using a nisin-inducible promoter. Specific Aim 4. Determine if Rgg binds to promoter regions of genes encoding exoproteins to control expression. Electrophoretic mobility-shift assays will be used to determine if Rgg binds to the promoter regions of mf-1 and speB.



**Grant:** 1R01AI052258-01A2  
**Program Director:** JACOBS, GAIL G.  
**Principal Investigator:** FRIEDMAN, JOEL M PHD  
**Title:** TrHbs: Biophysical Consequences of a Nonpolar Tunnel  
**Institution:** YESHIVA UNIVERSITY BRONX, NY  
**Project Period:** 2004/07/15-2008/06/30

DESCRIPTION (provided by applicant): Truncated hemoglobins (trHbs) are a recently discovered class of small oxygen-binding protohemeproteins, widely distributed in prokaryotes. Many occur in bacteria pathogenic to man (e.g. *Mycobacterium tuberculosis* (TB), *Mycobacterium avium*, *Mycobacterium leprae*, *Corynebacterium diphtheriae*, *Bordetella pertussis*, *Legionella pneumophila*, *Staphylococcus aureus* and *Bacillus anthracis*). This project will focus on trHbN and trHbO, the two trHbs from TB. TrHbN protects aerobic respiration from the inhibitory action of nitric oxide and enhances infectivity. The function of trHbO is essential for bacterial growth. Since trHbs are not present in man, this makes them potential drug targets. The emphasis of this project is on probing those unusual biophysical properties of these two trHbs that are hypothesized to be the basis for the unusual functionalities that are of potential biomedical significance. Several specific aims are designed to expose the nature and significance of the hydrogen bonding network found in the distal heme pocket and of the large apolar tunnel that links the solvent to the ligand binding site at the heme. To achieve in depth insight into how the hydrogen bonding network and the apolar tunnel functioning of these two important proteins, several techniques will be used including photolysis, stopped-flow spectrometry, vibrational spectroscopy (FTIR, UV and visible resonance Raman), structure analysis (X-ray diffraction, NMR), site directed mutagenesis and conformational trapping and kinetics of ligand rebinding. In parallel with this part of the overall project, genetic and molecular biological approaches will be used to correlate the biophysical results with the in vivo functional studies.

**Grant:** 1R01AI052289-01A2  
**Program Director:** PETERS, N KENT  
**Principal Investigator:** PEREGO, MARTA PHD  
**Title:** Signal Transduction in Enterococci  
**Institution:** SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA  
**Project Period:** 2003/12/15-2008/11/30

DESCRIPTION (provided by applicant): Enterococci are Gram-positive constituents of the human microflora and play beneficial roles in artisanal cheese production and as probiotics to treat gastroenteritis. Enterococci have emerged as one of the leading causes of nosocomial infections. The problem of enterococcal infection has been aggravated by the emergence of multiple antibiotic resistance that poses a serious challenge to therapeutic intervention. This is an issue of concern in medicine, to the scientific community and the general public. Direction in the search for new therapeutic approaches for treating enterococcal infections will come from a clearer understanding of the factors involved in the pathogenesis of the disease and their regulatory mechanisms. The availability of the *E. faecalis* genome sequence allowed us to identify the key components of the bacterial regulatory mechanisms carried out by two-component signal transduction systems. The research proposed in this application has the goal of defining the regulatory network of enterococcal signal transduction for the purpose of understanding the mechanisms underlying the physiology of these organisms. This will provide valuable information for the understanding of mechanisms responsible for pathogenicity and antibiotic resistance and thus the identification of molecular targets for therapeutic intervention. We have generated deletion mutants for all response regulators identified in *E. faecalis* V583. Phenotypes have been identified in some strains, and a role for the FsrA response regulator in controlling biofilm formation through regulation of gelatinase production has been revealed. We propose to: 1) determine the role of two-component systems in virulence through in vivo analyses; 2) determine the role of gelatinase in biofilm; 3) define the genes regulated by two-component signal transduction systems (the regulon) by transcriptional and proteomic approaches; 4) determine the role of the two-component systems in enterococcal physiology by turning ON or OFF the genes encoding response regulatory proteins and analyzing the resulting phenotypes.

**Grant:** 1R01AI052439-01A2  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** SCHOREY, JEFFREY S PHD  
**Title:** M. avium GPLs in macrophage activation and virulence  
**Institution:** UNIVERSITY OF NOTRE DAME NOTRE DAME, IN  
**Project Period:** 2004/03/15-2009/02/28

DESCRIPTION (PROVIDED BY APPLICANT): Mycobacterial infections result in the activation of macrophage signaling pathways that are essential for stimulating a host immune response. However our understanding is limited as to which signaling pathways are transduced in macrophages upon mycobacterial invasion and whether pathogenic mycobacteria modulate these signals. In our experiments to define the macrophage signaling pathways initiated during a mycobacterial infection we determined that the mitogen activated protein kinases (MAPK) p38 and ERK 1/2 were activated in murine macrophages upon mycobacterial infection. However, the MAPK activity was limited in macrophages infected with pathogenic M. avium strains relative to cells infected with non-pathogenic mycobacteria. A limited production of TNF-alpha was also observed in these M. avium infected cells. Inhibitor studies indicated that the MAPKs were required for the mycobacteria-mediated induction of TNF-alpha. Moreover, macrophages infected with a glycopeptidolipid (GPL) deficient M. avium 2151 also showed increased MAPK activation and TNF-alpha production compared to cells infected with a isogenic 2151 strain containing GPLs. Therefore, we hypothesize that the MAPKs are key components in the macrophage signaling pathways initiated during a mycobacterial infection and that M. avium has evolved mechanisms to limit their activity in part through production of GPLs. Additional analysis showed that modifying the GPL structure through deletion of the methyl transferase D gene resulted in a M. avium 104 strain with attenuated virulence. Thus, we propose the following specific aims: 1) produce a panel of M. avium 724 and 104 mutants that lack specific genes involved in GPL biosynthesis 2) Biochemically characterize the M. avium mutants for GPL structure and cell wall composition 3) Characterize GPLs for their affect on macrophage activation and mycobacterial virulence. Our long-term goal is to better understand the macrophage signaling pathways initiated during a mycobacterial invasion. We believe a more careful analysis of these macrophage responses and how mycobacteria may modify them will lead to novel insights into mycobacterial pathogenesis.

**Grant:** 1R01AI053069-01A1  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** KELLY, CIARAN P  
**Title:** Immune Response to Clostridium difficile  
**Institution:** BETH ISRAEL DEACONESS MEDICAL CENTER BOSTON, MA  
**Project Period:** 2004/04/01-2009/03/31

**DESCRIPTION** (provided by applicant): Clostridium difficile is the most common cause of infectious diarrhea in hospital patients and is associated with substantial morbidity, mortality and financial cost. The longterm goal of this project is to assist in the development and clinical application of novel non-antibiotic agents to prevent and treat C. difficile-associated diarrhea and colitis. The central hypothesis of this proposal is that host factors, especially the immune response to C. difficile antigens, play a major role in determining the clinical outcome of C. difficile infection. We will perform a prospective cohort study of 150 subjects with C. difficile-associated diarrhea (CDAD) to examine each of the following three hypotheses: Hypothesis 1: Immune responses to both C. difficile toxin A and toxin B are instrumental in protecting against recurrent CDAD. Our recent studies have shown that a serum IgG response to C. difficile toxin A is evident in subjects that are protected against symptomatic or recurrent CDAD. In this aim we will determine whether humoral and cell-mediated immune responses to toxin B, immune responses to toxin A recombinant peptides and/or toxin neutralizing activity are also associated with protection. Hypothesis 2: Risk for recurrent CDAD can be predicted based on clinical parameters and or anti-toxin antibody measurement. Based on our previous study of patients with CDAD (derivation cohort) we have developed prediction rules with >80% accuracy in predicting recurrence. In this aim we will prospectively test these rules in the 150 subjects recruited for Specific Aim 1 (validation cohort). Once validated these tools can be used in clinical practice and in research studies to identify high-risk patients that are most likely to benefit from measures to prevent recurrent C. difficile infection. Hypothesis 3: Immune responses to C. difficile Surface Layer Protein (SLP) can protect against colonization and/or CDAD. Previous studies of the immune response to C. difficile have focused almost exclusively on anti-toxin antibodies. C. difficile surface layer protein (SLP) is the predominant surface protein in C. difficile, has been characterized recently at the molecular level and appear to act as a bacterial adhesin. Our preliminary data indicate that an immune response to C. difficile surface layer protein may be protective. In this aim we will compare humoral and cell-mediated immune responses to purified and recombinant C. difficile SLP in subjects with a single episode of CDAD, those with recurrent disease and in disease and healthy controls. We will also determine whether vaccination against C. difficile SLP can prevent colonization by C. difficile and/or C. difficile associated ileo-cecitis in hamsters. These specific aims are designed to advance further our knowledge of the immunobiology of C. difficile toxin-induced diarrhea and the mechanisms of immune protection in humans.

**Grant:** 1R01AI053138-01A2  
**Program Director:** PETERS, N KENT  
**Principal Investigator:** CHANG, CHENG-WEI T PHD  
**Title:** Novel Ribostamycins and SAR Study of Ring III Aminosugar  
**Institution:** UTAH STATE UNIVERSITY LOGAN, UT  
**Project Period:** 2004/03/01-2009/02/28

DESCRIPTION (provided by applicant): Carbohydrate research can contribute profound impacts in various fields although the synthesis of carbohydrate derivatives has been the major obstacle that impedes the progress in this area. Through our experience and effort, we have achieved our preliminary projected goals: synthesis of aminosugars/deoxysugars library, preparation of aminosugars/deoxysugars containing molecules with practical applications, and the studies of biological implications with these aminosugars/deoxysugars containing molecules. We are one of the few groups capable of creating such diverse unusual sugar and aminoglycoside libraries. After successfully achieving our initial goal of generating aminosugar/deoxysugar library, we have moved into one of the envisioned applications of unusual sugars: synthesizing a library of pyranmycin, novel aminoglycoside antibiotics. Compared to previous work, our pyranmycin designs provide the only examples with antibacterial activity close to neomycin (low micromole), and with much-improved acid stability. The later may potentially lead to the reduction of the cytotoxicity known to be associated with aminoglycoside antibiotics. Using synthetic approaches, we intend to synthesize two more structural entities coupled with a solid phase parallel synthesis of oligopeptides for the development of the general aminoglycoside designs against various strains of resistant bacteria, and with lower cytotoxicity. These are very challenging but interesting and important tasks. We have proposed several practical synthetic methods for our objectives. We have established our own facilities or collaborations for evaluating the antibacterial activity of our pyranmycin constructs in vivo and in whole cell based. We have learned solid knowledge in carbohydrate synthesis and aminoglycoside antibiotic design through our research. We wish to learn more regarding the influence of modulating the interactions, charge-charge and van der Waals, and apply the finding for future drug designs. It is our conviction that our proposed research will provide breakthroughs in carbohydrate-related fields, and pave the way to a broader research ranging from basic scientific studies like carbohydrates and receptors interaction to practical applications regarding the issues of public health and national security, such as, the development of clinically important unusual sugar-containing antibacterial (against anthrax, MDR-TB), antiviral (against HIV), antifungal, and anticancer agents.

**Grant:** 1R01AI053191-01A2  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** PAOLETTI, LAWRENCE C PHD  
**Title:** Bacterial Vaccine Antigen Discovery  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 2004/04/01-2007/03/31

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

**Grant:** 1R01AI053459-01A2  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** SCHESSER, KURT R PHD  
**Title:** Cellular analysis of the Yersinia protein kinase A  
**Institution:** UNIVERSITY OF MIAMI-MEDICAL CORAL GABLES, FL  
**Project Period:** 2004/02/01-2009/01/31

**DESCRIPTION** (provided by applicant): The pathogenic yersiniae inject a number of virulence determinants, or effectors, directly into the host cell cytoplasm by the type III secretion system. This proposal focuses on uncovering the cellular activity of one of these type III effectors, the Yersinia protein kinase A (YpkA). We have observed that ypkA strains of Yersinia pseudotuberculosis are handicapped in proliferation- and antibiotic protection-based cell culture infection assays compared to the wild-type strain. YpkA is composed of a eukaryotic-like ser/thr kinase and GTPase-binding domains within its amino and carboxyl termini, respectively. An enzymatically inactive YpkA is attenuated in cell and animal infection assays but is fully competent in blocking phagocytosis. The latter activity appears to involve YpkA's GTPase-binding domain that has been previously linked to YpkA's cytoskeletal-disrupting activity. We believe that YpkA's proliferation-promoting and antiphagocytic activities are based on its ability modulate host cell functions that normally serve to limit microbial proliferation. Our working hypothesis is that YpkA's cellular activity is dependent on it physically contacting specific host proteins following its injection into the eukaryotic cell. To test this hypothesis, we are undertaking a three-prong approach in attempting to determine the basis of YpkA's cellular activity. (1) YpkA variants with specific deficiencies will be identified using various genetic, structural, and biochemical techniques. (2) Y. pseudotuberculosis mutant strains will be generated expressing the YpkA variants that come through our genetic and biochemical screens or identified in our structure-based analysis. And (3), proteomic analysis of host cells infected with the Y. pseudotuberculosis ypkA mutant strains will be performed in order to uncover YpkA-mediated changes in host cell protein phosphorylation activity. We are seeking to understand how YpkA enhances Yersinia's ability to withstand the microbial killing activity of the host cell, which in turn will undoubtedly provide key insights into YpkA's role in pathogenesis.

**Grant:** 1R01AI054193-01A2  
**Program Director:** LAMBROS, CHRIS  
**Principal Investigator:** MILLER, MARVIN J PHD  
**Title:** Design/Syntheses/Studies/Novel Antituberculosis Agents  
**Institution:** UNIVERSITY OF NOTRE DAME NOTRE DAME, IN  
**Project Period:** 2004/02/15-2008/01/31

DESCRIPTION (provided by applicant): Design, syntheses and studies of novel anti-tuberculosis agents based on biologically essential mycobacterial iron sequestration processes are proposed. Assimilation of iron is essential for most living organisms. Thus, microbes, including *Mycobacterium tuberculosis*, have evolved very selective and specific methods to sequester physiologically essential iron. The general hypothesis of this proposal is that the iron sequestration process utilized by *Mycobacterium tuberculosis* can be exploited as an "Achilles' heel" for the development of novel antituberculosis agents. Though this concept has been considered, no laboratory has previously been able to synthetically access the relevant compounds for related studies. The specific aims are the following. 1. Design, syntheses and studies of focused sets of analogs of natural iron chelators (mycobactins) used by *M. tuberculosis* to determine if analogs can inhibit iron acquisition, thus, inducing microbe selective iron starvation and microbe death (confirmation of "Snow's Hypothesis"). The analog design will build on our preliminary findings that selective structural variation of mycobactins does produce novel antiTB agents. Thus, practical scale syntheses of lead compounds will be followed by focused structural modification. The synthetic work will be complemented by full chemical and physical characterization of samples, including determination of the iron binding affinity of the mycobactin analogs and conjugates as well as their ability to bind iron from media. 2. Syntheses and studies of a focused and limited set of mycobactin (siderophore)- antibiotic conjugates capable of selective microbe cell transport and drug delivery. All conjugates can be prepared in straightforward fashion (one to three steps) from our already synthesized lead compounds. 3. In Vitro and in vivo biological evaluation of samples for antituberculosis activity, growth inhibition or promotion of other selected mycobacteria and related studies, including gross toxicity, will be performed to determine the mode of action of new compounds with anti-TB activity. Taken together, these studies will determine the feasibility of developing new antituberculosis agents with a novel mode of action related to the required iron uptake processes needed by mycobacteria, including *M. tuberculosis*.



**Grant:** 1R01AI054423-01A1  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** SALAMA, NINA PHD  
**Title:** Genetic requirements of *Helicobacter pylori* infection  
**Institution:** FRED HUTCHINSON CANCER RESEARCH SEATTLE, WA  
CENTER  
**Project Period:** 2003/12/01-2008/11/30

DESCRIPTION (provided by applicant): *Helicobacter pylori* (Hp) chronically infect the human stomach of 50% of the population worldwide. Ten to 20% of those infected will eventually present with severe disease including ulcers and gastric cancers. Our working hypothesis is that Hp disease is a by-product of the interaction between bacterial factors necessary for establishing and maintaining infection and the resultant host defenses. Furthermore, this interaction is dynamic with the bacteria modifying the host and the host modifying the bacteria over decades of infection. To study this complex process, we utilize a mouse model of infection which recapitulates many aspects of human disease including a robust immune response that is unable to clear the infection and alteration of gastric gland architecture. Using this model we will characterize the function of known Hp virulence factors in vivo and perform a saturating screen for new Hp virulence genes. Such a screen has not been possible until recently due to lack of experimental tools. In Aim 1 we examine the in vivo role of the major secreted cytotoxin, VacA, which we were the first to demonstrate has a phenotype during mouse infection. In Aim 2 we describe a screen to identify additional Hp virulence genes. Here we take advantage of two tools we recently developed: an Hp transposon mutant library and a novel methodology we call MATT to monitor transposon mutants in a pool using our Hp cDNA microarray. We believe this screen has the potential to give us a global view of the Hp genetic requirements for establishing and maintaining infection. Finally, in Aim 3 we investigate the PAI, a group of virulence genes that have been recently shown to mediate a number of specific interactions with cultured cells. We describe experiments that address why no in vivo phenotype for this locus has been described to date and new experiments to measure a role for the PAI during mouse infection. Careful study of infection with mutants in vacA, the PAI genes and newly identified virulence factors have the potential to teach us a great deal about wild type infection by revealing processes that fail to occur in mutant infections. These processes likely contribute to the various diseases associated with Hp and may highlight potential therapeutic targets.

**Grant:** 1R01AI054476-01A1  
**Program Director:** PERDUE, SAMUEL S.  
**Principal Investigator:** RIKIHISA, YASUKO  
PHD  
MICROBIOLOGY:BACTERIOLOG  
Y  
**Title:** TYPE IV SECRETION & SIGNAL TRANSDUCTION IN EHRLICHIOSIS  
**Institution:** OHIO STATE UNIVERSITY COLUMBUS, OH  
**Project Period:** 2004/01/01-2008/12/31

DESCRIPTION (provided by the applicant): Human monocytic ehrlichiosis (HME) caused by *Ehrlichia chaffeensis* and human granulocytic ehrlichiosis (HGE) caused by *Anaplasma phagocytophila*, are emerging infectious diseases. These bacteria are fastidious obligatory intracellular bacteria that require repeated transmission between two vastly different hosts: vertebrates and invertebrates. How these bacteria enter and continue to thrive within hostile host milieu such as neutrophils or the tick vector is largely unknown. We recently characterized genes encoding a type IV secretion system (T4SS) and two component-regulatory system (2CRS) in HGE and HME agents. This proposal aims to understand the roles of T4SS and 2CRS in pathogenesis of HME and HGE. The specific aims of this project are as follows: 1. Examine the colocalization of T4SS proteins expressed by individual *E. chaffeensis* and *A. phagocytophila* organisms with endosome-lysosome markers and signaling molecules in human leukocytes during attachment, internalization, and subsequent proliferation, and in response to environmental stimuli. 2. Characterize expression of T4SS in ticks and the skin of mammals during tick transmission. 3. Identify T4SS effector or substrate molecules. 4. Characterize the 2CRS of *E. chaffeensis* and *A. phagocytophila* by expressing recombinant proteins and studying the phosphorylation of sensor kinases and response regulators in vitro, and in vivo under controlled sets of environmental conditions; by examining the effects of 2CRS (histidine kinase) inhibitors on phenotypes of *E. chaffeensis* and *A. phagocytophila*; and by studying genes regulated by 2CRS. 5. Construct novel mutants of *A. phagocytophila* and *E. chaffeensis* in which a predicted critical gene is disrupted, or conditionally silenced to determine the functions of T4SS and 2CRS. The data obtained from this study will provide a new perspective for understanding the dynamic signaling events between obligatory intracellular bacteria and their mammalian and tick hosts. This project will potentially provide a technical breakthrough in this field of research. The results may point to a potential target for new treatment and prevention of HME and HGE.

**Grant:** 1R01AI054515-01A1  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** RAMAMOORTHY, A PHD  
**Title:** Structure and function of antimicrobial peptides  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2004/03/01-2009/02/28

DESCRIPTION (provided by applicant): The development of new active therapeutic agents is of increasing importance as more bacterial strains resistant to existing conventional antibiotics are emerging. Natural antimicrobial peptides represent one successful form of chemical defense that eukaryotic cells use against bacteria, protozoa, fungi, and virus. Antimicrobial peptides kill bacteria by disrupting their membranes, and these peptides may be developed into a new line of defense against infectious diseases. The main goal of the proposed research is to understand the mechanism and selectivity of a human antimicrobial peptide, LL37, and its derivatives. A combination of structural, dynamics, and thermodynamic studies will be used to investigate the mechanism of membrane-disruption, and the important lipid-peptide and peptide-peptide interactions that determine the specificity for disruption of bacterial rather than eukaryotic membranes. The main analytical tool in these studies is solid-state NMR spectroscopy, using a set of techniques which are well-suited to atomic-level structural studies in non-crystalline membrane systems. We will obtain the following high-resolution structural information about the membrane-disrupting mechanism of antimicrobial peptides: (i) secondary structure; (ii) orientation relative to the membrane bilayer normal; and (iii) dynamics and mechanism of membrane-disruption. The data from these three types of measurements will be combined to obtain a detailed picture of the membrane-bound antimicrobial peptides. The experiments will employ a variety of solid-state NMR methods in order to measure chemical shift and dipolar coupling parameters. Antimicrobial peptides will also be characterized in solution (prior to membrane insertion) and in micelles using circular dichroism and solution NMR experiments. Differential scanning calorimetry, deuterium NMR, and phosphorous-31 NMR experiments will also be used to probe local as well as global changes in lipid motional dynamics upon interaction with the antimicrobial peptides. This proposal aims to further our fundamental understanding of the antimicrobial peptide function to develop a new class of peptides that are more potent and selective than LL37 or related peptides. These peptides have therapeutic potential as antibiotics and have particular importance for cystic fibrosis patients.

**Grant:** 1R01AI054540-01A1  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** BRAUNSTEIN, MIRIAM BS  
**Title:** Protein Secretion Pathways of Mycobacterium tuberculosis  
**Institution:** UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC  
HILL  
**Project Period:** 2004/01/15-2007/12/31

**DESCRIPTION** (provided by applicant): More than two million people die each year from tuberculosis and one third of the world's population is believed infected with Mycobacterium tuberculosis, the bacterium responsible for this disease. To enable development of new drugs and vaccines for tuberculosis, a thorough understanding of M. tuberculosis physiology and pathogenesis is required. Protein secretion pathways play an important role in bacterial pathogenesis. The long-term objective of this research is to define the protein export systems of mycobacteria and the role they play in M. tuberculosis pathogenesis. Mycobacteria are unusual in possessing two SecA homologues (SecA1 and SecA2). SecA is highly conserved throughout bacteria and is a central component of the general Sec-dependent transport pathway, which exports proteins containing amino-terminal Sec signal sequences. In mycobacteria, SecA1 is the essential "housekeeping" SecA while SecA2 is a non-essential accessory secretion factor. A deletion of the secA2 gene in M. tuberculosis attenuated the virulence of the organism in mice. This suggests that SecA2 exports virulence factors of M. tuberculosis. The investigators identified two antioxidants of M. tuberculosis as being secreted by a SecA2-dependent export pathway. This has led to the hypothesis that SecA2 is part of a virulence mechanism of M. tuberculosis to evade the oxidative attack of the host. Both of these antioxidants lack Sec signal sequences and the mechanistic basis of their export is unknown. The proposed research will characterize the roles of SecA2 in pathogenesis and in protein export. The specific aims of this proposal are the following: (1) Investigate the role of SecA2 and secreted antioxidants in M. tuberculosis pathogenesis by testing a  $\Delta$ secA2 mutant of M. tuberculosis in both macrophages and mice and in its ability to resist oxidative attack, (2) Identify proteins exported by SecA2, and (3) Characterize the basis of SecA2 function in protein export by identifying other proteins that act with SecA2. This study will expand our understanding of the proteins that contribute to M. tuberculosis virulence and the protein secretion pathways of M. tuberculosis. It may also help clarify the role of reactive oxygen species in the host response to M. tuberculosis. This research has the potential of facilitating development of new anti-tuberculosis strategies.

**Grant:** 1R01AI054544-01A1  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** RAM, SANJAY MD  
**Title:** Complement Activation on Neisseria meningitidis  
**Institution:** BOSTON MEDICAL CENTER BOSTON, MA  
**Project Period:** 2004/01/01-2008/12/31

**DESCRIPTION** (provided by applicant): Serogroup B *Neisseria meningitidis* infections are a major cause of morbidity and mortality worldwide. Complement (C) is important in combating neisserial infections. Individuals with C deficiency are predisposed to neisserial infections. Antibodies (Abs) that induce bactericidal killing protect against invasive disease. We have identified lipooligosaccharide (LOS) as a major C4b target when other than specific anti-capsular Abs are used for opsonization. Phosphoethanolamine (PEA) on heptose 2 (Hep2) of LOS is an important acceptor for C4 (based on our data that C4b binds LOS via amide bonds). In specific Aim 1 we will study the relative roles of PEA residues in the 3- and 6-position of Hep2 in accepting C4b, by comparing C4b binding to LOS on strains that differ only in the location of PEA (either the 3- or 6-position) on Hep2. We have determined that Hep1 chain substitutions influence the nature of the linkage (ester or amide) formed between C4b and LOS. We will extend these findings and study the effect of Hep2 hexose substitutions (the IgtG gene product) on C4b binding to LOS. In Specific Aim 2, we will examine the ability of the isoforms of C4 in human serum to bind to LOS in the context of whole bacteria. Two isoforms of C4 with differing biological activities, called C4A and C4B, exist in human serum. While C4B is ~3 times more hemolytically active, C4A engages its target via exclusively amide linkages and binds complement receptor 1 (CR1) more efficiently. The ability of bacteria opsonized with serum selectively deficient in C4A and C4B to bind to CR1 will be studied in a chinese hamster ovary (CHO) cell line transfected with a mutant CR1 molecule that contains only the C4b binding site. Finally, in specific Aim 3, we will examine whether bacterial targets for C4b binding are altered by mAbs against a repertoire of bacterial antigens, in an effort to understand why certain antigenic targets (such as class 4 protein) are not good bactericidal targets despite being recognized by C-fixing Abs. We will also determine the relative efficiency with which two mAbs that deposit C4b on to different targets elicit C-mediated killing, in an attempt to define the requirements for a vaccine candidate to elicit the most efficient bactericidal (or opsonic) response.

**Grant:** 1R01AI054546-01A1  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** SELLATI, TIMOTHY J PHD  
**Title:** CD14 AND THE IMMUNOPATHOGENESIS OF CHRONIC LYME DISEASE  
**Institution:** ALBANY MEDICAL COLLEGE OF UNION UNIV ALBANY, NY  
**Project Period:** 2004/03/15-2009/02/28

DESCRIPTION (provided by applicant): Lyme disease (LD), a tick-borne infection caused by the spirochetal bacterium *Borrelia burgdorferi*, is a multisystem chronic inflammatory disorder that affects the skin, joints, heart, and nervous system. Although many aspects of LD pathogenesis remain ill-defined, it is generally accepted that clinical manifestations result primarily from the host's local immune response to spirochetes in infected tissues. In vitro evidence suggests that the impetus for this local immune response is recognition of *B. burgdorferi*'s major membrane immunogens (i.e., lipoproteins) by the pattern recognition receptor (PRR) CD14, a glycosylphosphatidyl inositol-anchored protein on the surface of monocytes/macrophages and neutrophils. Subsequent interaction between lipoprotein-CD14 complexes and the transmembrane transducing element Toll-like receptor 2 (TLR2) triggers a signaling cascade resulting in secretion of pro-inflammatory cytokines; these events likely contribute to the tissue and end-organ damage associated with LD. Despite the primacy and importance of lipoprotein binding to CD14 for immune cell activation in vitro, the consequences of this interaction during natural infection are entirely unknown. The overall objective of this proposal is to eliminate this gap in our knowledge through characterization of CD14's role in recognizing *B. burgdorferi* and initiating appropriate host defenses during borrelial infection. Toward achieving our objective, we have observed that CD14-deficient C3H/HeN (disease-susceptible) mice infected with *B. burgdorferi* via *Ixodes scapularis* ticks develop more severe and persistent disease compared with their wild type counterparts. Furthermore, peritoneal macrophages from CD14-deficient mice produced more proinflammatory TNF-alpha and less anti-inflammatory IL-10 than cells from wild type animals. Complementary studies using C57BL/6 (disease-resistant) mice are underway. These counterintuitive results suggest that CD14 is in fact dispensable for the initiation of disease and raise the intriguing possibility that signaling through this PRR may instead serve to down-modulate potentially damaging inflammatory cascades and protect the host from persistent infection. Based upon our findings, we hypothesize that engagement of the CD14-TLR2 signaling pathway provides negative feedback responsible for modulating the intensity and duration of inflammatory responses to *B. burgdorferi*. This hypothesis will be tested through pursuit of the following Specific Aims: 1) define the in vivo role of CD14 in modulating the clinical course and severity of Lyme disease, 2) characterize the ability of *Borrelia burgdorferi*-initiated CD14 signaling to modulate TLR2 expression and 3) identify components of the CD14 signaling cascade responsible for regulation of TLR2 and downstream inflammatory responses. It is our expectation that completion of these studies will identify the full complement of CD14 function as it relates to the development and resolution of disease triggered by borrelial infection and determine whether CD14-TLR2 signaling is requisite for establishment of disease susceptible/resistant phenotypes in mice. The rationale underlying this work is the belief that a better understanding of the immune processes that contribute to LD pathogenesis will serve as the basis for translational research that will yield improved control strategies and therapeutics to combat this chronic inflammatory disorder.

**Grant:** 1R01AI054716-01A2  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** BLUMENTHAL, ROBERT M PHD VET  
MEDICINE:MICROBIOLOGY  
**Title:** Conservation and Adaptation of a Regulon Across Genera  
**Institution:** MEDICAL COLLEGE OF OHIO AT TOLEDO TOLEDO, OH  
**Project Period:** 2004/07/01-2007/06/30

DESCRIPTION (provided by applicant): A primary motivation for determining genome sequences of microbial pathogens is to understand the bases of their pathogenicity. Proper regulation of virulence genes can be as essential to pathogenicity as is possession of these genes, so predicting regulatory networks from genome sequences is a high priority. A plausible but poorly tested assumption underlies many of these predictions. Specifically, the presence in two species of both a conserved regulatory protein and of conserved target genes with candidate upstream binding sites is presumed to imply that their regulatory relationship has been conserved. The two major goals of this project are to test this bioinformatic assumption and, in so doing, better characterize a major bacterial regulatory network (regulon). The leucine-responsive regulatory protein (Lrp) is conserved among many Gram-negative bacteria, and in *E. coli* affects expression of as many as 400 genes. Recent evidence indicates that many of these genes are preferentially expressed during transition to stationary phase, and may play a role in pathogenicity in related organisms. Three hypotheses will be tested: \* The hypothesis that species with conserved Irp genes have conserved Lrp function, to be tested by determining whether Lrp levels vary comparably in these species, and by assessing the extent to which Lrp orthologs are interchangeable. The Irp genes to be tested are from *Proteus mirabilis* (98% identical to the *E. coli* protein) *V. cholerae* (92%), and *P. multocida* (75%). \* The hypothesis that species with highly-conserved Lrp orthologs show a conserved pattern of regulation, to be tested by using microarrays to analyze the effects of Irp mutation in *E. coli* O157:H7, *V. cholerae*, and *P. multocida*. *E. coli* O157:H7 Lrp is identical to that of *E. coli* K-12, but the former is a pathogen with ~25% more genes, some of which may belong to the Lrp regulon. \* The hypothesis that Irp mutations have analogous effects on the virulence of different pathogenic bacteria, to be tested by determining the effects of a Irp null allele in an animal model for *V. cholerae*.

**Grant:** 1R01AI054830-01A1  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** ZECHIEDRICH, E LYNN PHD  
**Title:** COMBATTING QUINOLONE ANTIMICROBIAL RESISTANCE  
**Institution:** BAYLOR COLLEGE OF MEDICINE HOUSTON, TX  
**Project Period:** 2004/03/15-2009/02/28

DESCRIPTION (provided by applicant): Quinolones are some of the most frequently used, broad-spectrum antimicrobial agents. Resistance to these drugs has become a critical public health problem. Mutations in the drug target topoisomerases that result in drug resistance are well documented. In addition, overproduction of the multidrug efflux pump AcrAB has been reported to result in quinolone resistance in clinical isolates of *E. coli*. Our long-range goal is to determine how bacteria respond to exposure to quinolone agents and to use this knowledge to design more effective treatments. A goal of the present proposal is to identify the genetic alterations that lead to quinolone resistance in clinical *E. coli* strains and to determine the interrelationship between levels and frequency of quinolone resistance, the mutations, and other patient covariates. The central hypothesis is that following quinolone treatment, mutations conferring resistance occur additively, beginning with mutations in the topoisomerases and ultimately including overproduction of AcrAB and that the most resistant isolates contain additional mutations, including another multidrug efflux pump. Preliminary data support these hypotheses. Overproduction of any multidrug efflux pump has far reaching therapeutic consequences; antimicrobial agents from multiple different categories, in addition to the quinolones, would be ineffective against these bacteria. With the combined basic, clinical, statistical, genomic, and bioinformatic expertise of the investigators and the size of our patient population, we are uniquely poised to carry out the following specific aims: (1) Identify and categorize genetic alterations that cause quinolone resistance in clinical isolates. We will use high through-put methods to detect mutations in the genes that encode quinolone resistance. Statistical methods will be used to analyze potential interrelationships between the mutations. (2) Perform a prospective analysis of patient data. We will: (a) use the high-throughput methods developed in specific aim 1 to determine the genetic alterations occurring in *E. coli* isolated from patients hospitalized in the Texas Medical Center compared to isolates from various consortia around the world; (b) perform genome typing; (c) analyze these data with respect to demographic and clinical data for the patients to determine the probable causes of quinolone resistance. Until we have a better understanding of the mechanisms used by bacteria to cope with drug pressure, we cannot design better inhibitors or control antimicrobial resistant infections.



**Grant:** 1R01AI054920-01A1  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** KAZMIERCZAK, BARBARA I MS  
**Title:** Molecular Basis for Pseudomonas Recognition of Epithelia  
**Institution:** YALE UNIVERSITY NEW HAVEN, CT  
**Project Period:** 2003/12/01-2007/11/30

**DESCRIPTION** (provided by applicant): *Pseudomonas aeruginosa* is an opportunistic gram-negative pathogen that causes acute, hospital-acquired infections as well as chronic disease in patients with cystic fibrosis. Though the organism is ubiquitous in the environment and possesses many virulence factors found in other gram-negative bacteria, it rarely causes disease in healthy hosts. Acute infection by *P. aeruginosa* usually occurs in the setting of pre-existing epithelial tissue damage; this is mirrored by increased bacterial adherence, invasion and cytotoxicity toward damaged epithelial monolayers in vitro. This grant proposal describes experiments to elucidate the molecular basis for *P. aeruginosa* interactions with epithelial cells. Its primary objective is to understand how pathogen-host cell interactions result in the activation of contact-dependent virulence factors such as the Type III secretion system. We have defined a novel set of *P. aeruginosa* mutants that can no longer interact with epithelial cells to promote internalization. The ability of these mutants to cause in vitro cytotoxicity will be directly assayed in Specific Aim #1, allowing us to identify a subset of mutants deficient for interactions required for Type III mediated virulence. These mutants will be tested in a murine model of acute pneumonia to confirm that these mutations also result in attenuated virulence toward the intact host. We also present preliminary data about two mutants, *ide-2* and *ide-9*, which demonstrates that we have already identified two putative *P. aeruginosa* proteins required for epithelial cell interactions leading to Type III dependent virulence. Specific Aims #2 and #3 discuss in detail the characterization of these two mutants. The characterization of bacterial molecules and signaling pathways underlying host cell recognition and contact-dependent virulence in *P. aeruginosa* will provide novel targets for treating and preventing *Pseudomonas* colonization and disease. Such insights are likely to prove applicable to other gram-negative pathogens of animals and plants, which similarly activate Type III secretion of bacterial effectors required for virulence in response to poorly understood signals that follow host cell contact.

**Grant:** 1R01AI055008-01A1  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** LAUTENBACH, EBBING MD  
**Title:** Clinical Impact of Quinolone-Resistant E.coli carriage  
**Institution:** UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA  
**Project Period:** 2004/04/01-2008/03/31

**DESCRIPTION:** (provided by applicant) The emergence of resistance to the fluoroquinolone (FQ) class of antibiotics is an issue of great concern. Although FQ resistance is particularly worrisome for *Escherichia coli*, the most common gram-negative pathogen, the epidemiology of FQ-resistant *E. coli* (FQR-EC) has not been well described. Based on existing data, one may conceptualize three stages in the biological evolution of FQR-EC in a given individual: 1) acquisition of new FQR-EC fecal colonization; 2) persistence of FQR-EC fecal colonization; and 3) development of clinically apparent FQR-EC infection. The primary reason to conceptualize different stages of the evolution of FQR-EC is to increase the sensitivity in identifying critical components of the pathway that can be interrupted or manipulated clinically. Failure to recognize the distinct importance of these stages has limited the ability of past efforts to elucidate the epidemiology of FQR-EC because risk factors for the various stages likely differ, and because not all forms of FQR-EC colonization are equally likely to progress to infection due to differences in underlying FQ resistance mechanisms. Focusing on the progression from FQR-EC colonization to FQR-EC infection is critical for several reasons: 1) although *E. coli* colonizing the gastrointestinal (GI) tract serve as the primary source of *E. coli* that subsequently manifest as clinical infection, not all individuals colonized with FQR-EC develop FQR-EC infection; and 2) specifically isolating those risk factors that are associated with progression from colonization to infection are vital given the ultimate goal of identifying modifiable factors that will limit the occurrence of clinical FQR-EC infection. To date, risk factors for FQR-EC infection in patients colonized with FQR-EC have not been investigated. Investigating risk factors for prolonged FQR-EC fecal colonization is also essential for several reasons: 1) it is likely that the longer a patient remains FQR-EC colonized, the greater the likelihood of developing FQR-EC infection; and 2) humans are a major reservoir for *E. coli* and the GI tract may serve as the primary source from which FQR-EC can disseminate through the population. Risk factors for prolonged FQR-EC colonization have not been studied. The primary aims of this study are 1) to identify risk factors for progression from FQR-EC fecal colonization to clinical FQR-EC infection; and 2) to identify risk factors for prolonged FQR-EC fecal colonization. The primary hypotheses are that outpatient antimicrobial use and presence of efflux pump overexpression (as a mechanism of FQ resistance) are associated with both clinical FQR-EC infection and prolonged FQR-EC colonization. Elucidation of forces driving the emergence of clinical FQR-EC, particularly the relationship between clinical risk factors and FQ resistance mechanisms, is critical if efforts to limit the clinical impact of FQR-EC are to be successful.

**Grant:** 1R01AI055352-01A1  
**Program Director:** NEAR, KAREN A.  
**Principal Investigator:** HORWITZ, MARCUS A MD  
**Title:** Novel Antimicrobial Agents Against M. tuberculosis  
**Institution:** UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA  
**Project Period:** 2004/07/15-2008/10/31

DESCRIPTION (provided by applicant): M. tuberculosis is the world's leading cause of death from a single infectious agent and the leading cause of death in AIDS patients. The emergence of multidrug resistant tuberculosis poses a major threat to the public health, giving new urgency to research aimed at combating this ancient scourge. Moreover, multidrug resistant strains of M. tuberculosis (MDRTB) are a potential weapon of bioterrorism, and such strains have been classified as NIAID/CDC Category C Bioterrorism Agents. Studies proposed in this grant application build upon advances made in collaborative efforts between the Horwitz laboratory at UCLA and the Griffith laboratory at the U. of Wisconsin over the past two years to develop novel antimicrobials against M. tuberculosis for treatment of drug resistant organisms. During the past several years, the Horwitz and Griffith laboratories have laid the groundwork for the development of a new antimicrobial strategy against M. tuberculosis - targeting M. tuberculosis glutamine synthetase (GS). Thus, Horwitz et al. demonstrated that M. tuberculosis GS is a promising antimicrobial target, and that the high production of this enzyme is correlated with pathogenicity in mycobacteria and with the presence of a poly-L-glutamate/glutamine structure in the cell wall of pathogenic mycobacteria. Horwitz et al. showed further that inhibition of GS with L-methionine-SR-sulfoximine (MSO) inhibits M. tuberculosis growth in cell-free culture, in human macrophages, and in vivo in guinea pigs challenged by aerosol with M. tuberculosis. In combination with ascorbate, MSO is almost as potent as isoniazid, the leading antituberculous drug. Importantly, working in a collaboration during the past year, the Horwitz and Griffith laboratories have identified analogs of MSO that are highly potent against M. tuberculosis in vitro but lack certain drawbacks of MSO. This application has two major goals: 1) Develop novel MSO analogs that are better drug candidates than MSO because they are a) more selective for glutamine synthetase (GS) vs gamma-glutamylcysteine synthetase (gamma -GCS); and/or b) less well taken up into the brain where MSO exerts its major toxicity in sensitive species; and/or c) even more selective for M. tuberculosis GS vs. mammalian GS, and test the toxicity of the analogs in mice. 2) Test the novel MSO analogs for their capacity to inhibit M. tuberculosis growth in broth culture, in human macrophages, and in guinea pigs.

**Grant:** 1R01AI055651-01A1  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** FREITAG, NANCY E PHD  
**Title:** Host-pathogen Genetics Using *Listeria* and *Drosophila*  
**Institution:** SEATTLE BIOMEDICAL RESEARCH INSTITUTE SEATTLE, WA  
**Project Period:** 2004/07/01-2009/06/30

**DESCRIPTION** (provided by applicant): *Listeria monocytogenes* is a facultative intracellular bacterial pathogen that is an important agent of serious human food-borne infections. *L. monocytogenes* has served for decades as an outstanding model system for elucidating cellular and molecular interactions that take place during host infection. This proposal seeks to combine the power of bacterial genetics with the use of a genetically tractable model host system to isolate host mutants with altered resistance to *L. monocytogenes* infection. The fruit fly *Drosophila melanogaster* has been intensely studied as a model genetic system for decades, and it offers several advantages as a model host including the striking conservation of its innate immune recognition pathways with those of vertebrate animals. Studies have demonstrated the conservation of pathogenic mechanisms used by infectious agents within flies and vertebrates, and the feasibility of screening thousands of flies to isolate mutants with altered responses to infection provides a powerful means of identifying host factors that contribute to host survival. Recent work has demonstrated that *D. melanogaster* serves as a suitable host for *L. monocytogenes* infection, thus this proposal seeks to exploit the use of these genetically tractable organisms to functionally identify critical factors of both pathogen and host that contribute to the establishment of microbe infection. In Aim 1, experiments will examine the cellular course of *L. monocytogenes* infection within insect tissue culture cells, larvae, and adult flies. These studies will provide a foundation for the functional analysis of pathogen and host gene products identified for their potential roles in influencing the outcome of microbial infection. Aim 2 will functionally characterize *L. monocytogenes* mutants that are attenuated for virulence in flies. Bacterial gene products required for insect infection will be analyzed for potential roles in mammalian infection. Aim 3 will isolate and identify *Drosophila* mutants with altered susceptibility to *Listeria* infection. These studies will help identify the host factors that contribute to immune responses directed against intracellular pathogens. The ultimate goal of the experiments described will be the functional characterization of bacterial factors that support survival within the host, and the elucidation of host mechanisms that serve to counter bacterial survival strategies.

**Grant:** 1R01AI055710-01A1  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** BLANCHARD, THOMAS G PHD  
**Title:** H. PYLORI- REGULATORY T CELLS THAT LIMIT HOST RESPONSE  
**Institution:** CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH  
**Project Period:** 2004/02/01-2009/01/31

DESCRIPTION (provided by applicant): *Helicobacter pylori* (*H. pylori*) colonizes the human gastric mucosa and plays an etiologic role in the development of gastritis and peptic ulcer disease. Infection persists for life despite the induction of histologic gastritis and specific immune responses. Similar observations have been made in the *H. pylori*-mouse model. However, mice lacking either IL-10 or NADPH oxidase develop inflammation in response to *H. pylori* that is significantly more intense than infected wild type mice, and spontaneously clear the bacteria from the gastric mucosa. Additionally, eradication of *H. pylori* from immunized mice following challenge is also accompanied by more intense inflammation. Therefore, *H. pylori* may persist due to the inability of the host to develop sufficiently intense inflammation during infection. The induction of down-regulatory T-cells that prevent aberrant responses to noninvasive bacteria in the colon has been described. These mechanisms may be conserved along the gastrointestinal tract and may be active in the gastric mucosa. This proposal will test the hypothesis that activation of T-cells at the gastric mucosa during *H. pylori* infection induces IL-10 producing regulatory T cells that suppress the inflammatory response, thus allowing for persistent infection. A correlate of this hypothesis is that vaccination effectively bypasses this down-regulation by activating T-cells in lymphoid tissue where the induction of IL-10 producing T-cells is not favored. We will address this hypothesis by: 1) Characterizing surface markers and cytokine profiles of gastric T cell from infected and immune mice to distinguish regulatory T-cells from protective T-cells. Flow cytometry and ELISA spot assays will be used to examine freshly isolated T-cells. 2) Identify the factors in the gastric mucosa that contribute to the induction of these regulatory cells. Transgenic mice and co-culture models will be used to explore the relationship of specific co-receptors and cytokines to T-cell activation in the stomach. 3) Investigate how regulatory T-cells interact with other cells to down-regulate inflammation. Regulatory T-cells will be studied in mice and in vitro to define the extent of their regulatory properties. These studies will increase our understanding of gastrointestinal immunoregulation and the design of better immunotherapies.

**Grant:** 1R01AI055743-01A1  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** MCSORLEY, STEPHEN J PHD  
**Title:** CD4 T Cell Response to Salmonella  
**Institution:** UNIVERSITY OF CONNECTICUT SCH OF FARMINGTON, CT  
MED/DNT  
**Project Period:** 2004/01/15-2008/12/31

DESCRIPTION (provided by applicant): The recognition of microbes by the innate and adaptive immune system can lead to the resolution of infection and development of long-lived immunity. Many microbial pathogens gain entry to the host by penetrating mucosal surfaces of the lung, intestine and genito-urinary tract. However, the induction of immune responses to microbial pathogens at mucosal surfaces is not well understood. The specific aims of the proposal are: Aim 1. To identify the cell types that present Salmonella antigens in vivo in order to test the hypothesis that lymphoid dendritic cells activate Salmonella-specific CD4 T cells. These studies will directly examine the presentation of a Salmonella encoded antigen in vivo, in order to test the hypothesis that lymphoid dendritic cells are responsible for activating Salmonella-specific CD4 T cells after oral infection. Aim 2. To examine Salmonella-specific T cell activation in the spleen and define mechanisms that account for T cell unresponsiveness in this organ. Our preliminary data indicate that Salmonella-specific CD4 T cells are inefficiently activated in the spleen, despite bacterial replication in this organ. We hypothesize that the location of bacteria in the spleen red pulp physically separates antigen from Salmonella-specific T cells. This will be tested using a novel Salmonella-specific TCR adoptive transfer system. Aim 3. To examine CD4T cell differentiation and migration in order to test the idea that effector/memory T cells are not efficiently generated after Salmonella infection. Our preliminary data indicate a defect in non-lymphoid migration of Salmonella-specific T cells following oral infection. We hypothesize that T cell effector functions do not develop efficiently, due to the local mucosal priming environment. This issue will be tested by examining the effector cytokine production and non-lymphoid migration of Salmonella-specific T cell after oral infection. These studies will provide new insight into the development of immunity to mucosal pathogens.

**Grant:** 1R01AI055912-01A1  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** WILKS, ANGELA PHD  
**Title:** Heme Oxygenase: Structure, Function and Pathogenesis  
**Institution:** UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD  
SCHOOL  
**Project Period:** 2004/02/01-2009/01/31

DESCRIPTION (provided by applicant): Pathogenic bacteria require iron for their survival and ability to cause infection. Heme comprises 90% of the iron available within the host. Therefore, understanding the mechanism of heme acquisition and iron release will provide the knowledge required for the development of new therapeutic targets. Both gram-negative and gram-positive pathogenic bacteria have evolved receptor mediated heme uptake systems by which they acquire iron. A key step in the process is the release of iron from the heme macrocycle by the action of heme oxygenase (HO). The specific aims of the proposal are to a) structurally characterize the soluble bacterial HO enzymes from *C. diphtheriae* (cd-HO), *N. meningitidis* (nm-HO) and *P. aeruginosa* (pa-HO). The cd-HO is structurally homologous to the mammalian HO proteins and will serve as a model system for the larger membrane bound proteins. The nm-HO and pa-HO represent a new unique class of HO enzymes that in the case of pa-HO show an altered regioselectivity. Using both X-ray crystallographic and NMR methodologies we will be able to obtain significant insight into the role of protein conformation and dynamics in heme reactivity in this unique family of enzymes; b) elucidate the mechanism of heme hydroxylation and regioselectivity by a combination of site-directed mutagenesis and spectroscopic studies designed to identify key structural and electronic factors in determining both regioselectivity and the formation of a key intermediate in the HO reaction; c) To elucidate the mechanistic formation and biophysical properties of verdoheme and biliverdin. The elucidation of the mechanism of action of the bacterial HO proteins will be crucial in understanding the role of heme utilization in pathogenesis, as well as in future development of inhibitors as potential therapeutic agents.

**Grant:** 1R01AI055943-01A1  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** BARRY, EILEEN M. PHD  
**Title:** Conserved pneumococcal protein live-vector vaccine  
**Institution:** UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD  
SCHOOL  
**Project Period:** 2004/06/01-2008/05/31

**DESCRIPTION** (provided by applicant): The objective of this research plan is to test the hypothesis that three conserved protein antigens from *Streptococcus pneumoniae* can induce protective responses when delivered via a live attenuated *S. Typhi* vector strain. The use of protein antigens conserved on all pneumococcal serotypes as vaccine components has attracted a great deal of attention as a promising alternative to capsular polysaccharide-based vaccines do to the potential of inducing broad spectrum immunity against diverse pneumococcal serotypes. Three conserved pneumococcal proteins, including PsaA, PspA, and Ply have been shown to be immunogenic and to elicit protective responses in animal models; specifically, PsaA and PspA have been shown to reduce pneumococcal carriage while Ply and PspA protect against invasive challenge. Here we will test a novel delivery method by using live, oral, attenuated *S. Typhi* vaccine strains as delivery vectors expressing combinations of these critical antigens in a single formulation with which it might be possible to engender responses protective against both carriage and invasive phases of pneumococcal disease. In order to accomplish this, the three pneumococcal antigens must be expressed in optimal immunogenic conformation in the *S. Typhi* vector strain. Aim 1 will address optimization of expression of each individual antigen in *S. Typhi* by engineering plasmids directing protein expression in the optimal bacterial compartment, utilizing preferred promoters and expressing immunogenic fragments comprising protective epitopes. Aim 2 will utilize the optimal constructions of each individual antigen in engineering multi-antigen plasmids optimized for expression of all components. Aim 3 will evaluate the functional capacity of immune responses generated in mice immunized with *S. Typhi* derivatives expressing the pneumococcal antigens by utilizing two challenge models, one testing reduction in pneumococcal carriage and the second evaluating protection from lethal invasive challenge with wild type *S. pneumoniae*. A successful live vector-based protein antigen pneumococcal vaccine offers advantages as a needle free, inexpensive formulation capable of protecting against a broad range of pneumococcal serotypes. These studies will contribute knowledge of the protective capabilities of three pneumococcal protein candidate antigens individually and in mixtures, extend the use of *S. Typhi* live vector strains, and provide preclinical data necessary for advancing promising candidates into clinical trials.



**Grant:** 1R01AI055977-01A1  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** SEIFERT, H STEVEN  
**Title:** Gonococcal Pilus Structure and Function  
**Institution:** NORTHWESTERN UNIVERSITY CHICAGO, IL  
**Project Period:** 2004/02/01-2009/01/31

DESCRIPTION (provided by applicant): The Gram-negative bacterium *Neisseria gonorrhoeae* is the only causative agent of the sexually transmitted disease gonorrhea. The gonococcal (Gc) pilus is an important virulence factor of this human pathogen. Since Gc do not possess a Type II or Type III secretion apparatus, this Type IV pilus has an important role in host-pathogen interactions. The Gc pilus is involved in many aspects of gonococcal pathogenesis. First, the pilus functions in the initiating events of colonization by enhancing the ability of the bacterium to adhere to and interact with cells of the human host. Second, the pilus is involved in allowing the efficient transport of DNA into the bacterial cell for genetic transformation. Third, it is involved in twitching motility, a specialized form of locomotion expressed by all Type IV piliated bacteria. Much has been learned about the components that make up the Gc pilus assembly apparatus in the past several years, but how the pilus and its assembly apparatus act to promote adherence, DNA transformation, and twitching motility remains largely undefined. We propose a series of experiments to further our understanding of how the Gc pilus provides these functions for pathogenesis. In Aim 1 will utilize saturating mutagenesis to isolate loss-of-function and gain-of-function mutations in the secretin gene, PilQ. Loss-of-function mutations will define domains of PilQ required for expression, stability, and multimerization. Gain-of-function mutations will be screened for mutations that effect DNA transformation, antibiotic sensitivity, and twitching motility. Mutants will also be tested for changes in epithelial cell adherence. In Aim 2 will undertake a biochemical, yeast two-hybrid and biophysical analysis of three related predicted NTPases involved in orchestrating pilus function. These studies will provide new findings and insights into the structure and functions of the gonococcal pilus.

**Grant:** 1R01AI056215-01A1  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** NANO, FRANCIS E AB  
**Title:** A. Francisella tularensis pathogenicity island  
**Institution:** UNIVERSITY OF VICTORIA VICTORIA, BC  
**Project Period:** 2004/09/01-2008/05/31

DESCRIPTION (provided by applicant): Francisella tularensis is a highly infectious bacterium that is classified as a CDC Category A biological agent. The primary objectives of this proposal is to understand the role of gene products of a recently discovered "pathogenicity island" found in F. tularensis. The pathogenicity island was discovered by the applicants and collaborators, and found to contain genes needed for F. tularensis to grow in macrophages and cause disease in mice. An unusual feature of most of the genes of the pathogenicity island is that they were imported from a protozoa related to the causative agent of malaria. The experiments proposed in this application are designed to characterize the protein products of the pathogenicity island and to determine if some of the proteins play a role in disease that is similar to their counterparts in protozoan pathogens. Genetic and biochemical techniques will be used to characterize the expression pattern and the biochemical nature of the proteins encoded by the pathogenicity island, and microscopy approaches will be used to determine how the proteins affect macrophages infected by F. tularensis.

**Grant:** 1R01AI056286-01A1  
**Program Director:** ZOU, LANLING  
**Principal Investigator:** PASCUAL, DAVID W PHD  
**Title:** Mucosal Vaccines for Plague  
**Institution:** MONTANA STATE UNIVERSITY (BOZEMAN) BOZEMAN, MT  
**Project Period:** 2004/07/01-2007/06/30

DESCRIPTION (provided by applicant): Current vaccines for plague rely on formaldehyde-inactivated whole bacillus to stimulate antibody responses, which protects only against bubonic plague, but not the more lethal pneumonic form. In an attempt to develop a subunit vaccine for plague without the adverse reactions from the inactivated bacillus, more recent studies have defined two *Yersinia pestis* proteins that can confer 100% protection to both bubonic and pneumonic plague. However, efforts to vaccinate successfully via a mucosal surface have resulted in suboptimal stimulation of protective antibodies. To address this gap, we propose to adapt our DNA vaccine delivery systems to optimally stimulate protective antibodies to *Y. pestis* capsular protein, F1 antigen, and to a secreted protein, V antigen. We hypothesize that by vaccinating via a mucosal surface, we will enhance the stimulation of neutralizing secretory (S)-IgA and serum IgG antibodies to F1 and V antigens. Since illicit exposure to *Y. pestis* is believed to occur via an aerosol, it is essential that immune protection of the upper and lower airways is induced to protect against the more severe form of plague, pneumonic plague. To test our hypothesis, we propose two Specific Aims. Studies in Specific Aim 1 will adapt our M cell-targeted DNA delivery system, whereby the vaccinating DNA plasmid will be complexed to reovirus protein 1 coupled to polylysine and will be administered via intranasal (i.n.) or intratracheal (i.t.) routes. To obtain long-term memory responses, we will incorporate the molecular adjuvants, GM-CSF or the subunit A of cholera toxin or heat-labile toxin, along with the co-expression of a fusion protein between F1 and V antigens, and all within a single, bicistronic plasmid. The proposed studies will optimize a dose and route of a delivery regimen that best protects the host against pneumonic and bubonic plague. Studies in Specific Aim 2 will develop a vaccination regimen that combines the use of different M cell ligands to reduce vector neutralization with prime/boost strategies using different molecular adjuvants to stimulate long-lasting protective antibodies against *Y. pestis* challenge. From these studies, we will produce efficacious vaccines to plague, as well as a supporting vaccine regimen, that will confer protective immunity in the mucosal and systemic immune compartments.

**Grant:** 1R01AI056979-01A1  
**Program Director:** LAMBROS, CHRIS  
**Principal Investigator:** SCHOREY, JEFFREY S PHD  
**Title:** Macrophage signaling upon M avium infection  
**Institution:** UNIVERSITY OF NOTRE DAME NOTRE DAME, IN  
**Project Period:** 2004/03/01-2009/02/28

DESCRIPTION (provided by applicant): Mycobacterium avium is a major opportunistic pathogen in AIDS patients and a significant cause of increased morbidity and mortality in HIV infected individuals. M. avium is an intra-macrophage pathogen which requires attachment and invasion of its host cell to initiate disease. However, macrophages also play an essential role in controlling a M. avium infection. Therefore, a key aspect to the understanding of M. avium pathogenesis is to define the macrophage response to the mycobacteria and how the bacilli modulates this response to limit the macrophage's ability to control the infection. We have initiated studies to define the macrophage signaling pathways activated during a M. avium infection and how this differs between M. avium strains of varied pathogenicity and between pathogenic and non-pathogenic mycobacteria. The activation of these signaling pathways are necessary for the production of cytokines, chemokines and other effector molecules required to control a mycobacterial infection. Our studies have shown that macrophages infected with pathogenic M. avium show limited activation of the mitogen activated protein kinases (MAPK), cyclic AMP and other signaling molecules relative to cells infected with non-pathogenic mycobacteria. The consequence of this tempered signaling response is limited production of TNF-alpha, IL-1 beta and nitric oxide synthase by the M. avium infected macrophages. Therefore, we hypothesize that M. avium has evolved mechanisms to minimize the activation of the MAPK and other signaling molecules and that this ability is an important aspect of its pathogenicity. The glycopeptidolipids (GPL) are one well-characterized component of the M. avium cell wall and our recent data indicate that macrophages infected with a M. avium 2151 morphotype which lacks GPLs has prolonged MAPK activation compared to cells infected with a 2151 morphotype containing GPLs. Therefore we propose to: 1) Define the mechanism by which M. avium limits MAPK activation in infected macrophages 2) Determine the importance of GPLs in M. avium pathogenesis and in macrophage activation. We will use a combination of genetic, immunological and cell biological approaches to address these important questions.

**Grant:** 1R01AI057433-01A1  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** SULLAM, PAUL M. MD  
**Title:** Prophage-Encoded Binding of *S. mitis* to Human Platelets  
**Institution:** NORTHERN CALIFORNIA INSTITUTE RES & SAN FRANCISCO, CA  
EDUC  
**Project Period:** 2004/08/01-2009/01/31

**DESCRIPTION** (provided by applicant): The binding of streptococci to human platelets is a postulated central mechanism in the pathogenesis of infective endocarditis. Bacterium-platelet binding may be important both for the initial attachment of blood-borne organisms to the valve surface, and for the subsequent formation of macroscopic vegetations. Our previous research has shown that platelet binding by *Streptococcus mitis* strain SF100 is mediated in part by two cell wall-associated proteins (PblA and PblB) that are encoded by a temperate bacteriophage (SM1). Expression of both proteins on the bacterial surface is required for maximum levels of platelet binding by SF100. The overall goal of this proposal is to delineate further the mechanisms by which these proteins contribute to platelet binding, and to determine the role of PblA and PblB mediated binding in the pathogenesis of endocarditis. We will first purify PblA and PblB, and examine the binding properties of each protein with human platelets in vitro, to determine whether either or both proteins can bind human platelets directly in vitro. Formal binding analysis will be done to determine whether binding resembles a receptor-ligand interaction. Purified PblA and PblB will also be used to identify their respective platelet binding sites, by far western blotting and by immunoprecipitation or affinity chromatography. Platelet membrane proteins bound by either PblA or PblB will then be identified by several methods as needed (e.g., N-terminal sequencing, or mass spectroscopy). We will also assess the mechanisms for the export of PblA and PblB to the bacterial surface, and whether these proteins form platelet-binding complexes with other phage structural proteins. To address the role of these adhesins in the pathogenesis of endocarditis, we will compare the virulence of SF100 and selected mutants in a rabbit model of endocardial infection. By characterizing streptococcal adhesins for platelets, this research will further define the role of platelet binding in the pathogenesis of endocarditis. In addition, it may identify novel targets for new preventative or therapeutic strategies.

**Grant:** 1R01AI057443-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** SCHNAPPINGER, DIRK PHD  
**Title:** Alternative Respiratory Chains of *M. tuberculosis*  
**Institution:** WEILL MEDICAL COLLEGE OF CORNELL UNIV NEW YORK, NY  
**Project Period:** 2004/02/01-2008/01/31

DESCRIPTION (provided by applicant): About a third of the world's population is infected with *Mycobacterium tuberculosis* (MTB). In a stand off that may last decades during the period of clinical latency, a population of MTB persists in a state of apparent bacteriostasis until the host's ability to restrict growth of the pathogen is reduced by declining cell-mediated immunity. Then bacterial replication can resume and reactivation of latent foci leads to clinical disease in about 10% of the immune-competent individuals infected with MTB. Drug therapy of active TB takes 6 to 9 months. Premature termination of therapy decreases its success rate and leads to the development and spread of drug resistant and multi-drug resistant MTB. Drugs for treating active TB are relatively ineffective against MTB in the latent phase of the infection and against non-replicating MTB. New drugs that are active against non-replicating MTB might shorten drug therapy of active TB and also allow the treatment of latently infected individuals that are at high risk to develop active TB. Respiration is fundamental for growth of most bacterial species and also for survival during bacteriostasis. Respiratory chains that occur in MTB but not in humans might be suitable targets for the development of novel anti-mycobacterial drugs that are active against persisting as well as growing bacteria. The goal of this project is to determine the importance of these (alternative) respiratory chains for MTB pathogenesis. We will determine how the energy metabolism of MTB adapts to environments encountered within the host with experiments that monitor the expression levels of genes encoding respiratory enzymes. Using transposon mutants we will test whether alternative respiratory chains are important for pathogenesis of MTB in mice. We will also investigate whether respiratory chains with a low bioenergetic efficiency are important for the pathogen's ability to metabolize highly reduced carbon sources like fatty acids and whether anaerobic respiration is essential for MTB to survive hypoxia.

**Grant:** 1R01AI057472-01

**Program Director:** BAKER, PHILLIP J.

**Principal Investigator:** FISCHETTI, VINCENT A PHD  
MICROBIOLOGY:BACTERIOLOG  
Y

**Title:** Isolaton of new phage enzymes to kill B. anthracis

**Institution:** ROCKEFELLER UNIVERSITY NEW YORK, NY

**Project Period:** 2004/06/01-2009/05/31

DESCRIPTION (provided by applicant): Our current defense against *Bacillus anthracis*, and other bacterial bioweapons, is based primarily on the use of antibiotic therapies. This plan is flawed considering the possibility that the bacteria used in such weapons may express naturally occurring or engineered resistances to current therapeutic or prophylactic antibiotics. For this reason we are currently developing a novel class of antibacterial agents. Our system is based on the use of phage lysins to provide a rapid and specific killing action against bacterial pathogens of interest, in particular *B. anthracis*. In addition to offering a previously unavailable method of bacterial killing, phage lysins are primarily attractive in that bacterial resistance to their action cannot be detected, even after extensive attempts. Our laboratory is the first to use these enzymes in their purified form to kill colonizing pathogenic bacteria on mucous membrane surfaces and in blood. The enzymes are specific for the species or strain from which the enzymes were derived, indicating that these enzymes may be used for targeted killing of only the pathogenic bacterium with little to no effect on normal flora bacteria. During these studies we discovered that enzymes with two different specificities for cell wall bonds (i.e., amidase and muramidase) have a synergistic effect in their killing capacity. In our studies with the PlyG phage enzyme from the gamma phage that is specific for *B. anthracis*, we show that this enzyme is able to kill anthrax bacilli in vitro, reducing 10<sup>8</sup> bacteria to sterility in two minutes. In vivo, we are able to protect animals from lethal challenge with both a closely related bacillus to *B. anthracis* as well as *B. anthracis*. Because of the synergistic effects in these enzymes, this application is designed to identify and develop a combination of enzymes for *B. anthracis* that attack the four different bonds in the bacillus cell wall. This will ensure a more efficient killing action as well as reduce the possibility of the development of resistance to these enzymes. Phage enzymes will be isolated from phage found in the environment and phage lysogenizing *B. anthracis*. These enzymes will then be characterized as to their specificity, purified and used in both in vitro and in vivo systems to determine efficacy. Because these enzymes may be an important line of defense against an attack with drug-resistant *B. anthracis*, having a number of enzymes at our disposal may allow for better decisions as to their use if necessary.

**Grant:** 1R01AI057512-01A1  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** DARBY, CREG B BS  
**Title:** Yersinia pestis biofilms on C. elegans  
**Institution:** UNIVERSITY OF ALABAMA AT BIRMINGHAM BIRMINGHAM, AL  
**Project Period:** 2004/07/01-2009/06/30

DESCRIPTION (provided by applicant): The goal of this research is to understand the mechanism by which the bubonic plague bacterium, *Yersinia pestis*, colonizes its vector, the flea. This proposal describes an experimental system in which the nematode *Caenorhabditis elegans* is a surrogate for the flea, which allows use of powerful model organism genetic methods. *Y. pestis* colonizes the flea digestive tract and physically blocks the insect from feeding, leading to transmission of plague to new hosts through flea bites. This process requires the bacterial genes known as hms. *Y. pestis* creates a visible biofilm on the surface of *C. elegans*, which also requires hms genes. The biofilm blocks feeding of the nematodes and impairs their growth, a physical effect similar to the feeding blockage that occurs in the digestive tracts of infected fleas. These observations suggest that *Y. pestis* blocks fleas with a biofilm. The nematode will be used to further understand this process in an easily manipulated laboratory system, and the results can then be investigated directly in flea-bacteria interactions. The impact on human health of these studies will be: 1) Identification and characterization, in bacteria or flea or both, of molecular targets for drugs that could reduce or eliminate plague in rodent flea populations and be used in response to bioweapon attacks with plague-carrying fleas. 2) Deeper understanding of how biofilms attach to living tissues, a process important in a wide variety of infectious diseases. 3) Identification of nematode surface components, with potential for translation to treatment of helminthic infections. The specific aims of this proposal are: 1) Determine the roles in biofilm formation of hms and other *Yersinia* genes. 2) Determine the polysaccharide content and structure of the biofilm and develop reagents for its detection. 3) Clone three *C. elegans* gene involved in biofilm adherence and determine the localization in the worm of the encoded proteins.



**Grant:** 1R01AI057576-01  
**Program Director:** PETERS, N KENT  
**Principal Investigator:** HOOPER, DAVID C  
**Title:** Mechanism and Spread of Qnr-Mediated Resistance  
**Institution:** MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA  
**Project Period:** 2004/01/01-2008/12/31

DESCRIPTION (provided by applicant): Mechanism and Spread of Qnr-Mediated Resistance. Quinolones are widely used antimicrobial agents because of their broad antibacterial spectrum, low toxicity, and reliable action against otherwise resistant pathogens. Bacterial resistance to quinolones, however, is increasing and has reached alarming levels in some parts of Europe and the Far East. Various chromosomal mutations contribute to this resistance. Plasmid-mediated resistance was long thought not to exist. We discovered a plasmid-encoded protein termed Qnr that protects DNA gyrase from quinolone inhibition. Qnr acts additively with chromosomal mechanisms for quinolone resistance, belongs to the pentapeptide repeat family of proteins and, by a gel displacement assay, binds to the gyrase tetramer as well as to the GyrA and GyrB subunits with differing affinities. Although initially found at a single hospital in the United States, the qnr gene has recently been discovered in clinical *Escherichia coli* isolates from the Far East and in about 10% of quinolone resistant *Klebsiella pneumoniae* strains from the US. The aims of this proposal are to explore the hypothesis that Qnr blocks quinolone binding to gyrase, to study Qnr binding kinetics by surface plasmon resonance, to investigate whether Qnr can also protect gyrase from such protein inhibitors as MccB17, CcdB, and Gyrl, to study further the prevalence of qnr and the genetic basis of its acquisition by plasmids, and to explore whether Qnr and an active gyrase fragment can co-crystallize for structural analysis by x-ray diffraction. These studies are important not only for understanding an emerging resistance mechanism but should also reveal details of how DNA gyrase and related topoisomerases function and how proteins in the pentapeptide family interact with and regulate the activity of other proteins.

**Grant:** 1R01AI057585-01  
**Program Director:** PETERS, N KENT  
**Principal Investigator:** EARHART, CATHLEEN A PHD  
**Title:** Structural Biology of Gram Positive Virulence Factors  
**Institution:** UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN  
**Project Period:** 2004/06/01-2009/05/31

DESCRIPTION (provided by applicant): Gram-positive pathogens such as *Staphylococcus aureus*, *Streptococcus pyogenes* and *Enterococcus faecalis* are responsible for a number of deadly diseases. The ubiquitous *S. aureus* is a leading cause of hospital acquired infections (over 700,000 annually); *S. pyogenes* is the organism responsible for severe Group A streptococcal infection which has mortality rates in excess of 60%; and *E. faecalis* has become an important health concern as a mediator of the spread of antibiotic resistance and a leading cause of nosocomial infections. During the last decade widespread emergence of antimicrobial resistance in these organisms has been recognized as one of the major health threats for mankind. New antimicrobial agents are urgently needed to avert a coming plague of multi-drug resistant gram-positive pathogens. The long term objective of our work in this area is to provide a structural foundation for understanding how gram positive organisms cause disease. Such an understanding can lead to the development of new pharmaceutical agents and vaccines. The pathogenicity of staphylococci, streptococci and enterococci is a result of a remarkable array of virulence mechanisms by which they attack their hosts. These mediate: (1) adherence to host cells and tissue, (2) evasion of host defenses, (3) invasion of host tissue, and (4) dissemination of antibiotic resistance. Previously, we determined the structures of a number of toxin superantigenic virulence factors, including toxic shock syndrome toxin-1 and exfoliative toxins A and B from *S. aureus* and streptococcal pyrogenic exotoxin A from *S. pyogenes*. Here we propose to determine the structures of five virulence factors and relevant physiological complexes representative of the four key virulence mechanisms: PrgX, a pheromone response regulator; PrgB, an aggregation substance protein found in pheromone-mediated conjugative plasmids, streptococcal C5a peptidase which targets the chemotactic anaphylatoxin C5a; a SspC, a protease inhibitor specific for a major staphylococcal secreted cysteinyl protease, SspB; and, bhemolysin, a sphingomyelinase. Of the 5 targets chosen, 4 have already yielded diffraction-quality crystals.

**Grant:** 1R01AI057588-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** LIEN, EGIL PHD  
**Title:** The Role of LPS and Toll-like Receptors in Plague  
**Institution:** UNIV OF MASSACHUSETTS MED SCH WORCESTER, MA  
WORCESTER  
**Project Period:** 2004/03/01-2009/02/28

**DESCRIPTION** (provided by applicant): The Gram-negative bacteria *Yersinia pestis* is the causative agent of plague, and is classified as an NIAID category A priority biodefense agent. *Y.pestis* contains a well-described type III secretion system that has the ability to repress the host responses to the bacteria. However, less is known about immune activation by outer membrane components such as lipopolysaccharide (LPS), how these components interact with the host immune system, and their role in disease progression. Our goal is to define the role of *Y. pestis* LPS in the development of plague, and furthermore to characterize the impact of interactions by *Y.pestis* and its LPS with host Toll-like receptors (TLRs) and CD14 during the course of the disease. TLRs and CD14 are central in the innate immune response to microbial challenge. It has recently been suggested that *Y.pestis* produces a lipid A (main biologically active component of LPS) of lower potency when grown at 37 degrees C (host temperature) compared to 27 degrees C (flea temperature). Our hypothesis is that temperature induced alterations in *Y. pestis* LPS enable the bacteria to blunt responses mediated by TLR4, contributing to the diminished innate immune responses following infection. We will isolate lipid A from *Y. pestis* strain KIM (for which the genomic sequence is known) grown at 27xC and 37xC, and characterize the detailed structures. Furthermore, we will analyze the immune activation ability of *Y. pestis* (grown at 27 degrees C and 37 degrees C) and its LPS with an emphasis on interactions with the TLR signaling pathways, and relate structures to immune activation potential. To investigate the specific role of LPS in disease, we will make bacteria expressing a highly active LPS at 37 degrees C, by over-expressing LPS biosynthesis genes from *E.coli*. We will also generate mutants over-expressing the *Y.pestis* genes, and make bacteria deficient in the same genes. These bacteria will produce altered LPS at both 27 degrees C and 37 degrees C, and we will characterize LPS structure and cell activation potential. To establish the role of *Y. pestis* LPS in infection in vivo, we will test wild-type and the genetically modified *Y. pestis* for ability to mount immune activation and infection in wildtype mice and mice genetically deficient for TLRs, MyD88 and CD14. The completion of these studies will provide new information on how *Y. pestis* interacts with central elements of the innate immune system, knowledge that would be helpful in the development of new therapies for plague and related infections.

**Grant:** 1R01AI057652-01  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** OSBORNE, BARBARA A  
**Title:** Polyclonal human antibody in cloned HAC-transgenic cows  
**Institution:** UNIVERSITY OF MASSACHUSETTS AMHERST AMHERST, MA  
**Project Period:** 2003/12/01-2006/11/30

DESCRIPTION (provided by applicant): Enterotoxins are considered a possible bioterrorism threat because of its lethality and its durability. Staphylococcal and streptococcal enterotoxins include a group of toxins known as pyrogenic toxin superantigens. Exposure to a high dose of these superantigens can be fatal while exposure to lower doses is not fatal but can be quite debilitating. It has been suggested that botulinum toxin SEB are the two most important toxin threats on the battlefield. Exposure to SEB is thought to be major risk because of potential lethality as well the incapacitating effects SEB has on exposed individuals. Several nations have had bioweapons programs in the past that produced large quantities of enterotoxin bacteria. No mass vaccination program is envisioned for these toxins. In the absence of a vaccine, passive immunization with intravenous immunoglobulin (IVIG) of people exposed to bacterial enterotoxins has been suggested however the lack of a large and identifiable pool of such individuals makes this approach impractical. This proposal is to develop a large animal system for producing human polyclonal antibody against an enterotoxin peptide. It has been shown that transgenic mice carrying a artificial human chromosome (HAC) produce human antibody of all classes and with a broad repertoire when challenged with antigen. In the current work, a similar strategy will be applied to cattle, where the yield of antibody would be far greater than with mice. We have already shown that we can create cattle clones that have a HAC containing the human Ig genes, and that the chromosome is stable and is expressed throughout fetal development into neonatal life. Work is proposed to characterize the immune response to enterotoxin in normal and in cloned transgenic calves. This novel and practical solution to the limited supply of human IVIG has several advantages. First, it would enable the production of large quantities of human antibody at a reasonable cost. Second, it would provide greater flexibility in designing immunization strategies for producing high titer; high specificity antibody beyond what is possible with human volunteers. Third, it would provide a new enabling technology for producing clinically important human antibody reagents against other bacterial and viral pathogens.

**Grant:** 1R01AI057744-01A1  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** CHAPMAN, EDWIN R PHD  
**Title:** Receptors for clostridial neurotoxins  
**Institution:** UNIVERSITY OF WISCONSIN MADISON MADISON, WI  
**Project Period:** 2004/08/01-2009/07/31

DESCRIPTION (provided by applicant): Botulism was first described almost 200 years ago. This disease is caused by the botulinum neurotoxins (BoNT), which are seven related toxins (A-G) produced by toxigenic strains of *Clostridium botulinum*. These toxins are the most poisonous substances known. They act by entering neurons and cleaving proteins that mediate the exocytosis of neurotransmitters, resulting in paralysis and death. BoNTs are thought to bind to the surface of neurons via a double-receptor mechanism in which the receptor is a complex composed of gangliosides and protein(s). Identification of the toxin receptors, and the pathways that mediate entry, might provide a means to block the action of these toxins. Recent evidence indicates that members of the synaptotagmin family serve as the proteins components of the BoNT/B receptor. Synaptotagmin I and II exhibit distinct abilities to bind and mediate entry of BoNT/B. The first Aim of this proposal explores these differences with the goal of relating the structure of synaptotagmin with its ability to function as a toxin receptor. The precise mechanism by which the other BoNTs gain entry into cells is not known. Therefore, in the second Aim we will identify the pathways through which other BoNTs enter target cells. Our preliminary data indicate that different toxins enter cells via distinct pathways. In the third Aim we will further explore the means of BoNT host recognition and entry by identifying the receptor(s) that mediate internalization of BoNT/E. We have focused on this serotype because we have demonstrated feasibility using a BoNT/E affinity matrix; the long term goal is to identify receptors for additional BoNTs. Finally, in the fourth Aim we will develop FRET-based sensors that can be used to monitor BoNT activity in vitro and in living cells in real time. These sensors will make it possible to carryout high throughput screening to identify small molecules that can antagonize the action of the toxins. BoNTs are currently being used clinically to treat a variety of muscle dystonias and are also produced on large scales as potential biological weapons. The studies proposed here will provide new insights into the molecular mechanism of action of the BoNTs, and may provide a novel means to prevent poisoning by these substances.

**Grant:** 1R01AI057808-01A1  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** LILLARD, JAMES W PHD  
**Title:** Role of RANTES in Pneumococcal Immunopathogenesis  
**Institution:** MOREHOUSE SCHOOL OF MEDICINE ATLANTA, GA  
**Project Period:** 2004/08/01-2008/05/31

DESCRIPTION (provided by applicant): Streptococcus pneumoniae infections are becoming increasingly difficult to manage due to the inability of susceptible individuals to mount appropriate anti -polysaccharide and, to a lesser degree, -surface protein antibody responses as well as increasing antibiotic resistance. Hence, new prophylactic interventions and understanding of pneumococcal immunopathogenesis are greatly needed. This proposal stems from and focuses on our recent findings that RANTES (regulated on activation, normal T cell expressed and secreted) significantly, yet differentially, enhances mucosal and systemic immunity. We present preliminary data that RANTES mRNA mucosal expression is elevated during the primary inflammatory/adaptive recognition response to pneumococcal carriage, which suggests that RANTES is essential for protective mucosal immunity to S. pneumoniae infections. RANTES, MIP-1alpha, and CCR5 polymorphisms resulting in diminished expression are also associated with increased susceptibility to- and progression of- other mucosal pathogens in man. In this regard, our preliminary results show that RANTES blockade leads to the transition of pneumococcal carriage to lethal pneumonia in a mouse model of carriage, using S. pneumoniae strain EF3030. These findings provide the rationale to support the hypothesis that RANTES is essential for the induction of protective mucosal and systemic adaptive immunity against S. pneumoniae. We have emphasized in vivo approaches using mouse models of pneumococcal -carriage and -pneumonia to test this hypothesis. Aim One will assess the recognition phase host immune response to EF3030 challenge in normal, RANTES- or T cell- blocked mice. Aim Two will characterize the adaptive (activation/effector phase) mucosal and systemic immune responses to the phosphorylcholine determinant of C-polysaccharide (PC) and pneumococcal surface adhesin A (PsaA) during pneumococcal disease in control Ab-treated or RANTES-inhibited mice. Aim Three will ascertain the role of RANTES in protection against carriage and/or pneumonia induced by wild type, mutant surface protein (e.g., psaA-) and rough EF3030 strains. This study will provide important and new information regarding the cellular and molecular mechanisms that RANTES uses to induce protective immunity against pneumococci.

**Grant:** 1R01AI057870-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** BRADLEY, KENNETH A PHD  
**Title:** CHARACTERIZATION OF ANTHRAX TOXIN RECEPTOR INTERACTIONS  
**Institution:** UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA  
**Project Period:** 2003/12/15-2008/11/30

DESCRIPTION (provided by applicant): Virulence of *Bacillus anthracis*, the causative agent of anthrax, is associated with the activities of its secreted toxin. Anthrax toxin is comprised of a single cell-binding moiety, protective antigen (PA), and two catalytic moieties, lethal factor (LF) and edema factor (EF). LF is a protease that cleaves members of the MAP kinase kinase family of signaling proteins, and causes lysis of macrophages. EF is an adenylate cyclase that increases cellular levels of cAMP, disrupting water homeostasis and neutrophil function. PA binds the host cellular anthrax toxin receptor (ATR) and subsequently binds and translocates LF and EF into the host cytosol where they are active. Thus, the ability of anthrax toxin to exert its toxic effects on mammalian cells depends on the ability of PA to bind ATR. Very little is known about the natural function of ATR, and no natural ligands for this receptor have been reported. However, PA binds ATR through a structurally conserved integrin-like inserted (I) domain. I domains function as protein-protein interaction modules, and thus it is likely that host proteins that normally interact with ATR do so via the same domain to which PA binds. Identifying the non-toxin interactions that ATR is capable of engaging in and defining the binding requirements for both toxin and natural ligands will provide valuable information for the development of antitoxins that block toxin-receptor interaction while leaving normal ATR interactions intact. Therefore, the primary goal of these proposed studies is to elucidate the molecular requirements for binding to ATR. ATR ligands will be identified and ATR-PA and ATR-ligand interactions will be characterized. Comparative analysis between toxin and ligand interactions will be used to determine if the requirements for binding overlap or are distinct. Additionally phage display libraries will be screened for peptides that bind specifically to ATR. The information learned from these studies will be used to develop distinct classes of antitoxins based on soluble receptor, toxin or ligand mimetics, or small peptides that specifically block PA-ATR interactions.

**Grant:** 1R01AI057877-01A1  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** EICHENBAUM, ZEHAVAL PHD  
**Title:** Iron Acquisition From Host Proteins in *S. pyogenes*  
**Institution:** GEORGIA STATE UNIVERSITY ATLANTA, GA  
**Project Period:** 2004/05/01-2009/04/30

**DESCRIPTION** (provided by applicant): The long-term goal of this project is to increase the understanding of the physiology and virulence of the human pathogen *Streptococcus pyogenes*. This investigation is specifically concerned with the molecular mechanisms used by *S. pyogenes* to obtain iron during infection. Most bacteria require iron, which serves as a catalyst for electron transfer and is an essential component of many important enzymes. The vast majority of iron in the mammalian body is sequestered by high affinity proteins, resulting in an environment that is essentially free of unbound iron, which in turn presents a challenge to invading bacteria that need iron for growth. Heme and heme-compounds are valuable sources of iron for the hemolytic *Streptococcus*. Yet, the mechanisms used by *S. pyogenes* and related pathogens to capture and transport heme or iron are not well characterized. Genetic studies done in this laboratory identified two iron-regulated operons named *sia* (for Streptococcal Iron Acquisition) and *sit* (for Streptococcal Iron Transport) that are involved in the utilization of hemoglobin. The first specific aim dissects the function of the *sia* and the *sit* operons and their role in *S. pyogenes* physiology. Genetic and biochemical analysis that includes mutant characterization and employs in vivo binding and transport assays will be used. The second specific aim analyzes the operon role in virulence; studies will be conducted in zebrafish and mice infection models. The third specific aim analyzes the structure and function of surface receptors involved in hemoprotein utilization. Substrate recognition and heme or iron capture will be investigated by in vitro binding assays; site-directed mutagenesis and arbitrary PCR mutagenesis will be used to identify functional receptor domains. The interactions between different receptor components will be studied with solid phase binding assays, cross-linking, and immunoprecipitation. Iron acquisition from host hemoproteins is likely to have important implications on the physiology and virulence of *S. pyogenes*, an obligate human parasite. The proposed characterization of iron acquisition in *S. pyogenes* will advance our understanding of this important pathogen, and will add to the understanding of the process of iron uptake in other Gram-positive bacteria, many of which are important human pathogens.



**Grant:** 1R01AI057885-01  
**Program Director:** ZOU, LANLING  
**Principal Investigator:** CURTISS, ROY  
PHD  
MICROBIOLOGY:MICROBIOLO  
GY-UNSPEC  
**Title:** Attenuated Live and Recombinant *Yersinia pestis* Vaccines  
**Institution:** WASHINGTON UNIVERSITY ST LOUIS, MO  
**Project Period:** 2004/01/01-2008/12/31

DESCRIPTION (provided by applicant): *Yersinia pestis*, in three pandemics, resulted in some 200 million plague deaths and is still endemic throughout the world resulting in sporadic infections. Due to its inability to lead a saprophytic life and its residence in many rodent populations, plague is one of the most feared of zoonotic diseases caused by an obligate animal-human pathogen. The plague bacillus began to be used as a biological weapon at least 800 years ago and is today one of the more likely biological threats. Because of these considerations, we propose to: (i) Construct and evaluate recombinant attenuated *Salmonella typhimurium* vaccines (RASV) synthesizing *Y. pestis* K1M antigens in vivo after oral immunization of mice to identify antigens that stimulate protective immunity to challenge with virulent *Y. pestis* K1M. (ii) Construct and evaluate a recombinant attenuated *S. typhi* ISP1820 with an RpoS\* phenotype to deliver one or more protective *Y. pestis* K1M antigens. If it is found that different antigen delivery modes are required to induce protective immunity to *Y. pestis* challenge, we will construct all deemed necessary recombinant vaccines to be administered as a cocktail. (iii) Construct and evaluate a live attenuated *Y. pestis* vaccine that will induce enhanced immune responses to most, if not all, proteins used by *Y. pestis* to acquire iron, an important virulence attribute, and exhibit complete biological containment with no surviving bacterial cells after ten generations of growth due to an arabinose-regulated delayed cell lysis system. We will initiate our construction endeavors with *Y. pestis* KIM6+ that lacks the 70 kb Lcr plasmid and is totally avirulent and add back the Lcr plasmid after introducing attenuating mutations. We will also develop our Master File, prepare and fully characterize candidate vaccine Master Seeds for stability and safety, prepare and submit protocols for IRB approvals, submit information necessary to obtain INDs, and perform any other work needed to arrange that the best candidate vaccines be clinically evaluated in human volunteers.

**Grant:** 1R01AI057926-01A1  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** WANG, JULIA Y PHD  
**Title:** Dually Active Anthrax Vaccine Against Bacilli and Toxins  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 2004/07/01-2009/06/30

DESCRIPTION (provided by applicant): The overall goal of our study is to develop an effective, dually active anthrax vaccine (DAAV) that confers simultaneous protection against both the bacilli and the toxins. Systemic anthrax infection by *Bacillus anthracis* results in extensive septicemia, toxemia, and almost invariably death. The pathogenesis of anthrax involves two crucial processes, i.e., replication of bacilli and release of toxins. The bacilli protect themselves with a poly-gamma-D-glutamic acid (PGA) capsule to evade immune surveillance while secreting large amounts of toxins. Hence, we hypothesize that an effective strategy needs to both kill the bacilli and destroy the toxins. We further hypothesize that a DAAV can be constructed based on the two major virulence factors, specifically, the bacillus capsular PGA and the toxin core protective antigen (PA). Our preliminary study demonstrates that PGA-PA conjugates induce potent anti-PA and anti-PGA antibodies and protect immunized mice against lethal challenges with both anthrax toxin and analogous bacilli. Such vaccines are superior to existing vaccines that solely target anthrax toxins. The focus of our proposed study is to further develop and optimize PA/PGA-based DAAVs. We will prepare libraries of three classes of DAAV constructs (DAAV-X, E, and C) by systematically varying their structures. We will derive optimal constructs by comparing their ability to elicit anti-PGA and anti-PA antibodies and their ability to protect mice against challenges with lethal toxins and virulent bacilli. Optimized DAAVs will be tested for their efficacy in protecting mice and rabbits against anthrax spore challenges. Aim 1. To optimize "cross-linked" PGA-PA conjugates (DAAV-X). Aim 2. To create and optimize "end-linked" PGA-PA conjugates (DAAV-E). Aim 3. To develop combination anthrax vaccines consisting of mixtures of PA and PGA-based conjugates (DAAV-C). Aim 4. To evaluate the effectiveness of optimized DAAV candidates in various animal models of anthrax infection.

**Grant:** 1R01AI057927-01  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** SZTEIN, MARCELO B  
**Title:** Protective Immunity by Shigella vaccines in humans  
**Institution:** UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD  
SCHOOL  
**Project Period:** 2004/05/01-2009/04/30

**DESCRIPTION** (provided by applicant): The overall goal of the studies presented in this proposal is to identify the immunological mechanisms that mediate effective protection from shigellosis following vaccination and natural infection with *Shigella*. *Shigella* is a global infection that disseminates rapidly in settings where there is crowding and inadequate sanitation. Given the shortcomings of available public health measures to successfully control this infection, the appearance of drug resistance and concerns about its potential use in bioterrorism, the development of safe and effective vaccines to *S. dysenteriae* 1, *S. flexneri* 2a and *S. sonnei* is urgently needed. However, *Shigella* vaccine development has been hampered by a considerable lack of information of the specific determinants of protective immunity. Thus, the understanding of the immunological correlates of protection in humans to *Shigella* spp. is of great importance. Our working hypothesis is that both cell-mediated immunity and antibody responses play a central role in protection of volunteers from shigellosis following immunization with attenuated *Shigella* vaccine candidates or infection with wild-type *Shigella*. Specifically, using serum, stool and peripheral blood mononuclear cell specimens obtained from volunteers immunized with attenuated strains of *S. dysenteriae*, *S. sonnei* or *S. flexneri* 2a or challenged with wild-type *S. dysenteriae* (zlstxA strain), *S. sonnei* or *S. flexneri* 2a, we propose to test the following hypotheses: (1) secretion of interferon-gamma, and other cytokines to key *Shigella* proteins involved in cell invasion (e.g., IpaB, IpaC and IpaD) following immunization or challenge of volunteers with *Shigella* is mediated by CD4 + T cells and restricted by class II major histocompatibility complex molecules; (2) immunization or challenge of volunteers with *Shigella* elicits the appearance in circulation of specific cytotoxic T lymphocytes; (3) protective CMI against *Shigella* depends on a defined set of immunodominant epitopes derived from *Shigella* antigens; (4) immunization or challenge of volunteers with *Shigella* elicits the appearance in serum and stools of antibodies directed not only to LPS, but also to key molecules involved in *Shigella* invasion, e.g., IpaB, IpaC, IpaD and other *Shigella* proteins and (5) immunization or challenge of volunteers with *Shigella* elicits the appearance in circulation of (a) specific T and B lymphocytes expressing gut homing molecules (e.g., integrin alpha4beta7) and (b) expanded effector (Teff) and peripheral memory T cell (TEM) pools.

**Grant:** 1R01AI057932-01A1  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** REASON, DONALD C PHD  
**Title:** Structural determinants of human immunity to anthrax  
**Institution:** CHILDREN'S HOSPITAL & RES CTR AT OAKLAND, CA  
OAKLAND  
**Project Period:** 2004/06/01-2009/05/31

**DESCRIPTION** (provided by applicant): The recent, intentional dissemination of *Bacillus anthracis* caused significant morbidity and mortality, as well as widespread and costly disruption of essential public services. The resulting re-evaluation of current methods for the prevention and treatment of anthrax has revealed significant gaps in our knowledge of the basic immunobiology of this disease, and highlighted the need to develop methodologies for establishing vaccine efficacy in the absence of clinical trials. The research described in this proposal will define the molecular and structural characteristics of the protective antibody response elicited by the currently licensed human anthrax vaccine. Methods of repertoire cloning recently developed in our laboratory will be used to establish at the molecular level the structural diversity, variable gene usage, and somatic maturational history of the human antibody repertoire specific for the protective antigen (PA) of *B. anthracis*. The extent to which different individuals utilize the same immunoglobulin variable gene products to bind specific epitopes on the antigen will be determined. Through sequence analysis, the degree to which responding antibody clones have undergone somatic maturation will be ascertained. Clonally derived PA-specific binding domains will be expressed in vitro, the subset capable of blocking PA functional activity identified, and affinity and valence requirements for functionality established. Cloned antibody binding domains will be used to define the PA-associated antigenic epitopes recognized by the human immune response, and to pinpoint those PA-associated epitopes that elicit neutralizing antibodies. Our findings will be crucial for the rational design of PA subunit vaccines, and will aid in establishing in vitro correlates of protective immunity to *B. anthracis* infection. In addition, the antibodies isolated will constitute a panel of fully human monoclonal binding domains with potential for therapeutic use as passive immunogens.

**Grant:** 1R01AI057956-01  
**Program Director:** ROTHERMEL, ANNETTE L.  
**Principal Investigator:** WEAVER, CASEY T MD  
**Title:** Immune Regulation to Intestinal Bacterial Antigens  
**Institution:** UNIVERSITY OF ALABAMA AT BIRMINGHAM BIRMINGHAM, AL  
**Project Period:** 2004/04/01-2009/03/31

DESCRIPTION (provided by applicant): Regulatory T cells are a CD4<sup>+</sup> T cell subset with immunosuppressive activity that have been identified in humans and mice. These cells exert tolerance through a dominant mechanism that has obvious therapeutic implications for intervention in autoinflammatory diseases and transplantation. We have identified IL-10 producing CD4<sup>+</sup> T cells in the normal mouse intestine that are reactive to enteric bacterial antigens and have Treg function in vitro and in vivo. We speculate that this population develops from mature, naive CD4 T cell precursors that recognize enteric bacterial antigens, and that IL-10 can be a useful marker with which to identify and study these cells. In this proposal, we will make use of a novel antigen-specific model of colitis based on the DO11.10 TCR transgenic mouse to examine the origin, function and maintenance of intestinal Tregs. We will also employ a new IL-10 reporter knock-in mouse to provide a functional marker that can be used to identify, isolate and characterize IL-10 producing Tregs in the intestinal tissues. These results will form the basis for future comparative studies with Treg cells isolated from human intestinal tissues. The specific aims are to test three distinct, but related hypotheses: (1) IL-10 producing CD4 T cells (CD4<sup>+</sup>IL - 10<sup>+</sup>) reactive to commensal bacterial antigens are a naturally occurring Treg population that exist in the intestinal mucosae and are the principal population responsible for intestinal immune homeostasis; (2) intestinal CD4<sup>+</sup>IL-10<sup>+</sup> Tregs require the enteric flora for development and maintenance; and (3) intestinal Tregs suppress the development and maintenance of colitogenic effector T cells through bystander inhibition. These studies will advance our understanding of intestinal Tregs and will provide insights into how the immune system maintains tolerance to the enormous antigenic challenge represented by the enteric bacterial flora. Defective immunoregulation to the bacterial flora is a common feature of chronic intestinal inflammation in most murine models and is postulated to occur in patients with inflammatory bowel disease (IBD), such as Crohn's disease and ulcerative colitis. These studies will provide a basis for manipulation of Treg function as a therapeutic approach to restoring dysregulated T cell responses in IBD.

**Grant:** 1R01AI058009-01A1  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** KRAIG, ELLEN B  
**Title:** NOVEL APPROACH TO CHLAMYDIA VACCINE DEVELOPMENT  
**Institution:** UNIVERSITY OF TEXAS HLTH SCI CTR SAN SAN ANTONIO, TX  
ANT  
**Project Period:** 2004/05/01-2008/04/30

DESCRIPTION (provided by applicant): Chlamydia trachomatis is the leading bacterial cause of sexually transmitted disease (STD) in the United States and continues to spread in the population as >50% of the infected individuals show no overt symptoms and fail to obtain treatment. These untreated chlamydial infections pose a particular health risk in women by leading to severe complications including pelvic inflammation, tubal infertility, and ectopic pregnancy. Although a high priority, it has proven difficult to design an effective vaccine for Chlamydia trachomatis. It has been shown that T cells are necessary for immune protection, but they may also contribute to inflammation associated with pathogenesis. Thus, it is of great importance to delineate the roles of immune T cells during infection. Although T cell clones specific for C. trachomatis have been reported, it has been difficult to obtain sufficient numbers of such homogeneous lines to analyze the repertoire of the T cell response to this pathogen. Thus, we have exploited T cell hybridoma technology in order to generate panels of cloned helper (CD4 +) and cytotoxic (CD8 +) T cells from mice that had been vaginally inoculated with viable Chlamydia trachomatis. The T cell hybridomas will provide us with a unique tool for use in identifying the antigens from C. trachomatis that are capable of eliciting a broadbased T cell response in mice. In addition, through a new collaboration with clinical investigators at Wilford Hall Medical Center, Lackland Air Force Base, it will be possible to assess whether T cells from humans infected with Chlamydia respond to the same antigens that were identified in mice. These aims represent the first time that T cell hybridomas have been used to assess the activation of T cells in response to an infection with viable Chlamydia and should provide important insights into novel approaches for enhancing immunity to C. trachomatis and to other intracellular pathogens.

**Grant:** 1R01AI058024-01  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** KLUG, CANDICE S PHD OTHER AREAS  
**Title:** Site-directed spin labeling of ArnT  
**Institution:** MEDICAL COLLEGE OF WISCONSIN MILWAUKEE, WI  
**Project Period:** 2004/03/08-2009/02/28

**DESCRIPTION** (provided by applicant): The ability of bacteria to resist host defense mechanisms is a major contributor to the virulence of bacterial infections. Bacterial resistance to antimicrobial peptides that play a key role in early stages of infection is especially significant. The proteins and substrates involved in the ability of bacteria such as *Salmonella typhimurium* and *Escherichia coli* to develop resistance to antimicrobial peptides have recently begun to be identified based on genetic analysis. The most recently identified protein involved in polymyxin resistance is the gene product for an inner membrane protein, termed ArnT, which is responsible for transferring an aminoarabinose moiety onto lipid A, conferring upon the bacteria resistance to the cationic antimicrobial peptide polymyxin. Obtaining a more thorough understanding of structure-function relationships in ArnT will be key to developing strategies to overcome resistance to polymyxin and other cationic peptides. Previous studies of ArnT have all involved in vivo enzymatic activity and genetic analyses to determine its role in polymyxin resistance; the ArnT protein has not previously been purified and studied by any methodology. The goal of this proposal is to study the structure of the purified inner membrane protein ArnT by site-directed spin labeling (SDSL) EPR spectroscopy in order to provide the first structural information on this newly identified transferase. A model is proposed in which the *Salmonella typhimurium* ArnT transferase is comprised of twelve transmembrane ( $\alpha$ -helices; this model will become the basis for the structural evaluation of the novel protein ArnT by SDSL EPR spectroscopy followed by the examination of structural changes in ArnT due to substrate recognition. In order to begin providing the first structural information on ArnT, a unique and new membrane protein, the following points will be addressed using SDSL EPR spectroscopy: 1) create and characterize a reactive-cysteine-free construct of ArnT; 2) evaluate the model predicting that ArnT is comprised of twelve transmembrane  $\alpha$ -helices by nitroxide scanning through a putative transmembrane helical region; 3) explore the overall structural arrangement of ArnT by analyzing small sets of mutations placed within putative transmembrane, surface loop, and substrate binding regions; and 4) monitor local and global structural changes induced by substrate binding. It is anticipated that these studies will provide insights into the local and global structure of ArnT, a previously uncharacterized integral membrane protein, which is of fundamental importance in furthering our understanding of the structure and functional dynamics of membrane proteins.

**Grant:** 1R01AI058045-01A1  
**Program Director:** TSENG, CHRISTOPHER K.  
**Principal Investigator:** BEHAR, VICTOR PHD  
**Title:** Synthesis of Lactonamycin and Related Polyketides  
**Institution:** RICE UNIVERSITY HOUSTON, TX  
**Project Period:** 2004/07/01-2007/06/30

DESCRIPTION (provided by applicant): Lactonamycin was isolated by Matsumoto and coworkers in 1996 from *Streptomyces rishiriensis* in a screen for new antibiotics active against drug resistant bacterial strains. Lactonamycin exhibits potent antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE). Structurally related polyketides including the tetracenomycins and elloramycin display potent antitumor activity. The goals of this research program are as follows: 1) Development of general synthetic methods to access lactonamycin, elloramycin, saintopin E, and the numerous tetracenomycin structures. In particular the construction of the ABCD-ring systems through a strategy of tandem 1,4-addition-Dieckmann ring closure will provide access to the natural product targets as well as analogs. 2) Construct advanced synthetic intermediates postulated as biosynthetic precursors of lactonamycin as part of elucidating the biosynthetic pathway to lactonamycin. 3) Construct an appropriate form of the carbohydrate L-rhodinose for completion of a chemical synthesis of lactonamycin. 4) Expand and define the scope of the tandem 1,4-addition-Dieckmann ring closure for applications to related type II polyketide natural products. Further expansion of the methodology to the synthesis of condensed polycyclic heterocycles. 5) Expand the [3+2] cycloaddition reaction of quinones with nitrile oxides to gain access to diverse type II polyketide structures such as DNA helicase inhibitor heliquinomycin.



**Grant:** 1R01AI058052-01

**Program Director:** BAKER, PHILLIP J.

**Principal Investigator:** TEUSCHER, CORY BS VET  
MEDICINE:MICROBIOLOGY

**Title:** Genetics of Suscptibility to Anthrax Toxin in vivo

**Institution:** UNIVERSITY OF VERMONT & ST AGRIC BURLINGTON, VT  
COLLEGE

**Project Period:** 2004/03/01-2009/02/28

DESCRIPTION (provided by applicant): The major virulence factor of *Bacillus anthracis* is an exotoxin composed of three separate proteins: protective antigen (PA), edema factor (EF) and lethal factor (LF). In binary combinations, PA in association with EF forms edema toxin whereas PA in association with LF forms lethal toxin (LT), the principal toxin mediating lethal anthrax pathologies. In vivo studies using inbred strains of mice revealed that mortality is genetically controlled, and that macrophages mediate this response since macrophage-depleted mice are resistant to LT challenge. Genetic studies using differential in vitro susceptibility to LT-induced macrophage cytolysis mapped *Ltxs1*, the gene controlling this phenotype, to central mouse chromosome 11. Recently, a positional candidate gene cloning approach identified *Ltxs1* as *Kif1c*. However, genetic studies on mortality following direct challenge with LT or infection have received limited attention. To assess the role of *Ltxs1/Kif1c* in the genetic control of mortality following LT challenge, we carried out a congenic mapping study using a panel of interval-specific recombinant congenic lines carrying various segments of central chromosome 11 derived from LT resistant DBA/2J mice which were introgressed by marker assisted selection onto the LT susceptible BALB/cByJ background. The results of this study revealed that mortality elicited by LT challenge is controlled by three linked quantitative trait loci (QTL) on central chromosome 11: *Ltxs1/Kif1c*, *Ltxs2* and *Ltxs3*. Importantly, in order to recapitulate dominant resistance to mortality as seen in CD2 F1 hybrids, DBA/2J alleles are required at all three QTL. In this application, we propose to undertake a positional candidate gene cloning approach to identify the genes underlying *Ltxs2* and *Ltxs3*. Toward this end we will: 1) use high resolution congenic mapping to reduce the candidate intervals to a resolution of 1.0 cM or less, 2) establish DBA/2J and BALB/cByJ BAC contigs across the intervals, and 3) generate BALB/c-TgN(D2-BAC *Kif1c*), DBA/2-TgN(C-BAC *Kif1c*), BALB/c-TgN(CD11b-*Kif1cd*), and DBA/2-TgN(CD11b-*Kif1cc*) transgenic lines and assess their susceptibility to LT and macrophage cytolysis. This will serve as proof of principal that BAC and single gene transgenic mapping can be used to identify the *Ltxs2* and *Ltxs3* genes, and verify that *Kif1c* is the gene within the DBA/2J *Ltxs1* interval controlling mortality and macrophage cytolysis.

**Grant:** 1R01AI058080-01  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** LEE, KYUNG-DALL PHD  
**Title:** Mechanism of Listeriolysin O in Cytosolic Delivery  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2003/12/01-2007/11/30

**DESCRIPTION** (provided by applicant): The cytosolic space of cells is an important target for drug delivery systems and intracellular pathogens. Many drugs are membrane-impermeant because of their large molecular size and hydrophilic characteristics; therefore the need for their specialized, efficient cytosolic delivery strategy is greater than ever. *Listeria monocytogenes* enters endocytic compartments and utilizes the specialized mechanism of the pore-forming protein, Listeriolysin O (LLO), to breach the endosomal membrane to escape into the cytosol. LLO has been utilized in drug delivery systems and has demonstrated its powerful utility as an endosomolytic agent in generating non-viral/nonbacterial delivery systems that mimic the *Listeria* invasion to deliver exogenous macromolecules into the cytosol. The molecular mechanism of the necessary and sufficient agent, LLO, is still not clearly understood. Specifically, its pH-dependent activity and its molecular regulators, such as the required reduction of the unique single cysteine, are not clear at this point. The goal of the current grant proposal is to understand the key elements of LLO activity and its regulatory mechanisms, likely dependent on cell types and cellular differentiation and metabolic states, and to use this knowledge to better design cytosolic delivery and targeting strategies in the future. Key questions to be addressed are: (i) LLO interaction with cholesterol-containing membranes, its membrane-binding domain, pH sensitive elements, on/off rates of binding, oligomerization rate, and how and which domains of LLO regulate these processes, (ii) how the unique cysteine of LLO is reduced in the endocytic pathway to activate the LLO activity and how that is regulated, and (iii) how delivery strategies are affected by various regulatory factors of LLO. As the reduction of disulfide bonds in the endocytic compartment is essential in many cases of pathogenesis and drug delivery systems as much as in the regulation of LLO activity, a significant part of the proposal is dedicated to designing and characterizing probes to monitor the reduction in endosomes in general and to investigate cellular factors modulating the reduction processes. The results and information obtained from the proposed research will be extremely important and critical not only for the rational design of LLO-mediated macromolecule delivery and for the long-term strategy of efficient cytosolic delivery but also for any drug delivery strategy utilizing reversible disulfide bonds, as well as for the elucidation of the *Listeria* invasion mechanism.

**Grant:** 1R01AI058122-01A1  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** GRANOFF, DAN M MD  
**Title:** Molecular basis meningococcal group A capsular immunity  
**Institution:** CHILDREN'S HOSPITAL & RES CTR AT OKLAND, CA  
OAKLAND  
**Project Period:** 2004/06/01-2008/05/31

DESCRIPTION (provided by applicant): Group A meningococci cause massive epidemics of meningitis and sepsis in sub-Saharan Africa. Compared to other bacterial polysaccharides (PS), group A has a number of unusual properties including being highly immunogenic in infants, and priming for booster antibody responses. Also, depending on the age of the person, or antigenic stimulus (natural exposure to group A or cross-reacting organisms, or conjugated vs. unconjugated PS vaccination), the PS can elicit bactericidal or non-bactericidal group A anticapsular antibodies. The role of non-bactericidal group A anticapsular antibodies in protection against group A disease is unknown, and the molecular basis for differences in antibody functional activity are poorly understood. Our hypothesis is that differences in antibody avidity and/or fine antigenic specificity, dictated by the structure of the antibody paratope, underlie these disparities in antibody functional activity. In this proposal we will characterize naturally acquired and vaccine-induced group A anticapsular antibodies from persons of different ages living in North America or sub-Saharan Africa, two areas of the world with vastly different risks of exposure to group A meningococci. Passive antibody protective activity will be measured in an animal model of group A bacteremia that will be developed. To define the V region genes utilized by the human antibody response to group A PS, and to determine the extent of hypermutation, we will perform combinatorial repertoire cloning and expression library analyses of group A PS-specific Fab fragments. This approach will be complemented by limited amino acid sequencing of VH and VL regions of clonally purified anticapsular antibodies and determination of V region genes by mass fingerprint analysis by MALDI-TOF mass spectroscopy of H and L chains separated by 2D gels. Together, these studies will elucidate the molecular basis by which human antibodies recognize group A PS, and will identify the mechanisms underlying the age- and vaccine-related disparities in antibody protective activity. The results may lead to establishment of more reliable surrogates of protective immunity for assessment of the efficacy of new group A conjugate vaccines being developed for elimination of epidemic meningococcal disease in sub-Saharan Africa. Our proposed studies also will increase our knowledge of human antibody recognition of bacterial PS antigens in general, and explain why some anticapsular antibodies confer protection against encapsulated bacteria, while others do not.

**Grant:** 1R01AI058129-01A1  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** SELSTED, MICHAEL E MD  
**Title:** Physiologic Peptide Cyclization in Myeloid Cells  
**Institution:** UNIVERSITY OF CALIFORNIA IRVINE IRVINE, CA  
**Project Period:** 2004/06/01-2009/05/31

DESCRIPTION (provided by applicant): Defensins are tridisulfide peptides implicated in innate immunity against potentially pathogenic microorganisms. Myeloid defensins are packaged in the granules of neutrophils and monocytes, and epithelial defensins are expressed in a wide variety of mucosal tissues. The most recently discovered defensins, termed theta-defensins, are 18-amino acid macrocyclic peptides that are stabilized by three parallel disulfide bonds. Isolated from rhesus monkey leukocytes, theta-defensins are remarkably potent antibiotics that kill bacteria and fungi, and they inactivate HIV-1. Antimicrobial activity is abrogated by opening of the backbone ring. The presence of macrocyclic peptides in animals was not previously known. Moreover, the biosynthesis of theta-defensins is novel, as the cyclic peptide is synthesized from two 9-amino acid segments that are spliced together in a head-to-tail configuration. While the cellular machinery that mediates this post-translational pathway is unknown, we hypothesize that enzymes expressed in theta-defensin-producing cells are responsible for the nonapeptide excision and ligation steps necessary for biosynthesis of the mature cyclic molecule. We propose to characterize the molecular components of the theta-defensin processing pathway by pursuing three specific Aims: 1. In Specific Aim 1, we will analyze the pro-theta-defensin intermediates produced in myeloid cells, and will determine the subcellular compartments of the molecular intermediates identified. 2. Specific Aim 2 is to identify pro-theta-defensin converting activities in extracts of theta-defensin-expressing cells. For these studies we will use synthetic and recombinant forms of putative substrates involved in the excision/ligation pathway, and use immunoprecipitation, and chromatographic, electrophoretic, and mass spectroscopic methods for detecting and characterizing the relevant enzymatic activities. 3. Specific Aim 3 is to characterize proteins that interact with pro-theta-defensins and subsequent intermediates, as these are likely to be convertases or chaperones necessary for the excision/ligation steps involved in theta-defensin biosynthesis. Results obtained from these studies are likely to disclose novel mechanisms that have evolved for splicing and cyclizing proteins in mammalian cells.

**Grant:** 1R01AI058131-01A1  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** STEYN, ANDRIES JC PHD  
**Title:** WhiB3 in *M. tuberculosis* virulence  
**Institution:** UNIVERSITY OF ALABAMA AT BIRMINGHAM BIRMINGHAM, AL  
**Project Period:** 2004/06/15-2009/05/31

DESCRIPTION (provided by applicant): *M. tuberculosis* (Mtb) is one of the leading causes of death worldwide and claims millions of lives annually. Approximately 1.7 billion people worldwide are asymptotically infected with the tubercle bacillus and constitute a major impediment to worldwide public health control measures. Previous work had shown that a point mutation (Arg515->His) in the 4.2 domain of RpoV, the principal sigma factor in *Mycobacterium bovis*, is attenuating. Using the yeast two-hybrid system, we have established that the 4.2 domain of virulent Mtb specifically interacts with a regulatory protein WhiB3. In contrast, the attenuated RpoV allele containing the single point mutation was unable to interact with WhiB3. We constructed a Mtb whiB3 mutant (deltawhiB3) and showed that it behaved identical to the wild-type strain with respect to its ability to replicate in mice and guinea pigs in vivo. Mice infected with AwhiB3 showed significantly longer survival times than mice infected with the wild type Mtb. In addition, the lungs of AwhiB3-infected mice appeared much less adversely affected. It is notable that this virulence gene would not have been detected using conventional screens such as signature tagged mutagenesis, which screens for mutants primarily defective in growth, and not virulence. Furthermore, we have shown that a whiB3 mutant of virulent *M. bovis*, in contrast to AwhiB3, was completely attenuated for growth in guinea pigs. Mtb contain seven WhiB homologues that show strong homology to proteins that are critical for sporulation in *Streptomyces* spp. We hypothesize that WhiB3 regulates the expression of mycobacterial components that modulate the host immune system. To better understand the mechanism of whiB3 in Mtb virulence, we will use electron paramagnetic resonance spectroscopy (EPR) to biochemically characterize the WhiB3 Fe-S cluster genes, identify genes under WhiB3 control, and characterize proteins that interact with the WhiB family. We will also demonstrate that WhiB3 is a DNA binding protein capable of activating transcription of specific target genes. We will study the in vivo expression of the whiB family and their role in virulence. These studies will characterize the WhiB family as potential targets for interventions that may abolish virulence, but not growth. These studies will also provide insight into understanding whether TB is an anomalous immunological reaction in response to the persistent bacilli, whether the bacilli themselves induce lethal immunopathology, or if it is a combination of both.

**Grant:** 1R01AI058146-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** ZHOU, ZHAOHUI S PHD  
**Title:** Mechanism and Inhibition for LuxS: A Biodefense Target  
**Institution:** WASHINGTON STATE UNIVERSITY PULLMAN, WA  
**Project Period:** 2004/07/01-2009/06/30

DESCRIPTION (provided by applicant): Bacterial infections, particularly those emerging with antibiotic resistance, pose an alarming threat to public health. Our long-term objective is to identify, characterize and validate new antibacterial targets. Traditional antibiotics act by killing or inhibiting bacteria, hence inducing antibiotic resistance. It is therefore imperative to explore alternative or complementary approaches. In the past few years, the ubiquitous bacterial enzyme LuxS has been found to play diverse and pivotal roles in bacterial quorum sensing, virulence regulation, toxin secretion and biofilm formation. In addition, central metabolic roles for LuxS are also proposed. This enzyme is found in Category A pathogens, including *B. anthracis* and *Yersinia pestis*; and in Category B pathogens, including *Vibrio cholerae*, *Salmonella* and diarrheagenic *E. coli*. Absent in humans, LuxS is an attractive target for anti-infective agent development. The enzymatic mechanism of LuxS remains elusive. Based on our preliminary studies, we propose that LuxS possesses functions of both an aldose-ketose isomerase and a lyase. The dual function of LuxS is mechanistically intriguing. Our proposed mechanism involves an initial aldose-ketose isomerization to generate a ketone at the C3 position on the carbohydrate moiety, and a final beta-elimination to cleave the C-S bond in S-ribosylhomocysteine. Our Specific Aim 1 is to chemically synthesize the proposed intermediates and their analogs, and test them as LuxS substrates or inhibitors. We will also attempt to trap or directly observe the proposed intermediates. Our Specific Aims 2 and 3 are to investigate the catalytic roles of Glu57 and Cys84 in *B. subtilis* LuxS by mutagenesis and chemical rescue, and the biological relevance of Cys84 oxidation. Lastly, we plan to design, synthesize and test mechanism-based inhibitors for LuxS, particularly those interacting with the active site zinc ion. We will also investigate how halogenated furanones, a group of natural antibacterial agents, inactivates LuxS, particularly for the *V. cholerae* enzyme. Additionally, we will test the effects of LuxS inhibitors on quorum sensing, biofilm formation and related bacterial physiology.

**Grant:** 1R01AI058198-01A1  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** GULATI, SUNITA DSC  
**Title:** Mimotopes of gonococcal oligosaccharides for vaccination  
**Institution:** BOSTON MEDICAL CENTER BOSTON, MA  
**Project Period:** 2004/07/09-2009/06/30

DESCRIPTION (provided by applicant): Alternative strategies to the design of microbial vaccines are needed that take advantage of antigenic determinants that serve as good targets for an immune response, but are not themselves effective immunogens. For example, oligosaccharide (OS) determinants isolated intact from bacterial lipopolysaccharides (or lipooligosaccharides [LOSs]) are poor immunogens, usually resulting only in T-cell independent responses. We have approached the design of a potential vaccine candidate against *Neisseria gonorrhoeae* by "converting" an OS epitope derived from gonococcal LOS into mimics: (i) an anti-idiotope antibody and (ii) short peptides, each mimicking the oligosaccharide [OS] epitope. This epitope, called 2C7, was defined by its reactivity with a monoclonal antibody (mAb), also named 2C7. 95% of gonococcal strains display the LOS-derived 2C7 epitope in their LOS, in vivo, in infected patients. In addition to 2C7 epitope, we plan to convert a second commonly expressed OS epitope, called 2-1-L8, to a peptide mimic(s). Humans develop antibodies against both of these epitopes after natural infection. Antibodies directed against the 2C7 and 2-1-L8 epitopes both show strong complement dependent bactericidal function. To enhance specific immune responses, using 2C7 and 2-1-L8 as representative vaccine candidates, and also to characterize the parameters necessary to construct successful peptide mimics of saccharide antigens, we propose to make peptide/adjuvant constructs, test their ability to stimulate effective immune responses directed against the nominal (OS) epitope, and characterize and optimize antigen presentation and T-cell signaling by these constructs. In specific aim 1, we will conjugate peptide mimics of *N. gonorrhoeae* oligosaccharide (2C7 and 2-1-L8) to known adjuvants that will include: complement component C3d and alpha-2 macroglobulin, mannose and proteosomes, the latter comprised of gonococcal porin (Por) proteins. In specific aim 2, we will immunize mice intraperitoneally and intranasal with these peptide/adjuvant complex preparation and test immunogenicity by measuring IgM, IgG and IgA responses, against the nominal OS epitopes, elicited in serum and vaginal washes. We will also examine functional immune responses in vitro by bactericidal assay and protection in vivo, by assessing the prevention or shortening of gonococcal colonization in estradiol-treated mice. In specific aim 3, we will study the role of different antigen presenting cells (APCs) and the T-cell signals that they produce in the immunopotential of adjuvants on the immune response to peptide mimics. These studies will serve to characterize the parameters required to drive the immune response of peptides, used as OS mimics, to a specific response against the nominal epitope and also define methods necessary both to produce a T cell mediated antibody response and to define the resulting characteristics against peptides used as saccharide mimics.

**Grant:** 1R01AI058200-01A1  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** RUBIN, LORRY G  
**Title:** PCR assays to detect individual pneumococcal serotypes  
**Institution:** NORTH SHORE-LONG ISLAND JEWISH RES MANHASSETT, NY  
INST  
**Project Period:** 2004/05/01-2007/04/30

**DESCRIPTION** (provided by applicant): Background *Streptococcus pneumoniae* (pneumococcus) is a major human pathogen. Pneumococci carried in the pharynx are the reservoir for both transmission and infection. There are 90 antigenically distinct serotypes based on the polysaccharide capsule. Anti-capsular antibodies provide type-specific immunity. In 2000, a pneumococcal conjugate vaccine, a vaccine containing capsular antigens from seven serotypes that markedly reduces the incidence of invasive infections in infants, was licensed and is now given to all U.S. infants. Vaccine recipients have reduced carriage of vaccine serotypes but an apparent increase in carriage of other serotypes. Standard methods for growing pneumococci from respiratory specimens have a limited sensitivity and generally detect only a single serotype although carriage of more than one serotype is common. There is a need for more sensitive tests that detect all serotypes present in pharyngeal specimens. The broad, long-term objective of this project is to study the carriage of all serotypes of pneumococci and the effect of vaccines on carriage. Specific Aims To develop sensitive and specific PCR-based assays to detect DNA from 30 serotypes/serogroups that account for 99% of pneumococci in clinical specimens. To compare the sensitivity and specificity of these assays with standard culture and mouse injection for detection of all serotypes present in clinical pharyngeal specimens. Research Plan For each of 30 serotypes (or serogroups) that collectively comprise 99% of clinical isolates, serotype/serogroup-specific genes & sequences in the capsular polysaccharide biosynthetic cluster will be identified. Using these sequences sensitive PCR-based assays will be developed for each of the 30. The specificity of each assay will be tested using DNA from strains of the 90 serotypes & other bacteria that colonize the pharynx. The sensitivity for detecting all serotypes of pneumococci in respiratory specimens will be compared with standard culture & mouse inoculation. Significance These PCR-based assays will allow more sensitive detection of all serotypes present in respiratory specimens resulting in a more complete understanding of the epidemiology of colonization. They will be used to test the hypothesis that the prevalence of colonization with non-vaccine serotypes is similar in vaccinated and unvaccinated infants. Furthermore, they will be critical for determining the effects of current and future pneumococcal vaccines on serotype-specific carriage.



**Grant:** 1R01AI058253-01A1  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** SEGALL, ANCA M. PHD BIOLOGY  
**Title:** Novel antibiotics that trap Holliday junctions  
**Institution:** SAN DIEGO STATE UNIVERSITY SAN DIEGO, CA  
**Project Period:** 2004/06/01-2009/05/31

DESCRIPTION (provided by applicant): Our use of antibiotics is outpacing the rate at which bacterial pathogens become resistant to the currently available drugs. Meanwhile, the development of new antibiotics is being abandoned by large pharmaceutical companies to small biotech concerns and academic labs. While studying site-specific recombination, we have identified several hexapeptides that inhibit recombination reactions and bind to Holliday junctions, preventing their resolution. We have discovered that these hexapeptides have antimicrobial activity in both gram+ and gram- bacteria, although they are 4-8 fold more potent in gram+ bacteria (including methicillin resistant Staph aureus). Depending on the organism, MICs range from 8-64 mu/g/ml. The antimicrobial activity is associated with chromosome partitioning defects, filamentation, and induction of DNA breaks. Based on confocal microscopy with fluorescently labeled peptides, we have found that the peptides penetrate both gram+ and gram- bacteria. Despite the fact that they are also able to penetrate eukaryotic cells, we have not detected cytotoxic effects in HeLa cells at doses up to 250 mu/g/ml. Our short term goals are to understand the basis of the mechanism of action of these peptides in bacterial cells, to test their antimicrobial activity against intracellular pathogens, and their ability to cure bacterial infections in animal model systems. In addition, we will investigate their activities in different subcellular compartments of eukaryotic cells with an eye towards maximizing their specificity against bacteria and minimizing cytotoxic side effects. Since the peptides may not be optimal for pharmacological use, we are also identifying non-peptide lead compounds that mimic the activity of the peptides. The specific aims of this study are to: 1) Identify and characterize nonpeptide small molecules that trap Holliday junctions. 2 & 3) Investigate the bacterial targets of the Holliday-junction trapping compounds using a combination of biochemical and genetic approaches. 4) Investigate effects on bacterial membranes. 5) Investigate the effects of Holliday-junction trapping compounds on eukaryotic cells. As part of this specific aim, we will also determine whether the peptides kill intracellular bacteria. 6) Investigate the effect of the peptides on Salmonella and Staph infections in mice in two well-studied infectious disease model systems.

**Grant:** 1R01AI058284-01A1  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** THOMPSON, STUART A BS  
**Title:** Campylobacter jejuni outer membrane protein vaccine  
**Institution:** MEDICAL COLLEGE OF GEORGIA (MCG) AUGUSTA, GA  
**Project Period:** 2004/05/01-2009/04/30

DESCRIPTION (provided by applicant): Campylobacter jejuni is the leading cause of bacterial gastroenteritis in the U.S., and has been classified by the NIH as a Category B Bioterrorism Agent due to its ability to cause food-borne and water-borne outbreaks. There are at least 2.4 million cases of C. jejuni disease in the U.S. annually, with an incidence exceeding that of Salmonella and Shigella combined (5). C. jejuni infection is also the most common antecedent event to the development of Guillain-Barre Syndrome (GBS), an acute motor paralysis that apparently results from an autoimmune response directed against C. jejuni surface antigens. An effective vaccine against C. jejuni is therefore highly desirable, to protect the U.S. population from both naturally-occurring C. jejuni disease and that arising from potential bioterrorist attacks. Vaccines based on C. jejuni whole-cell preparations have been proposed, however, due to uncertainties concerning the development of GBS, alternative approaches are warranted. A vaccine consisting of highly conserved outer membrane proteins (OMPs) may therefore hold the most promise for safely inducing protective immunity without the potential for inducing GBS. It is well recognized that C. jejuni strains are highly variable, and this will certainly impact on the development of a protein subunit vaccine. A protein appropriate for vaccine inclusion must be conserved in the largest possible proportion of C. jejuni strains, must be immunogenic, and must induce protective immunity against a large number of diverse C. jejuni strains. While analysis of the C. jejuni genome sequence is helpful as a starting point toward understanding its complement of OMPs, only direct identification of OMPs (by proteome analysis) will provide detailed information about the OMPs actually expressed by C. jejuni strains. Hypothesis: Certain C. jejuni outer membrane proteins (OMPs) will be conserved among all C. jejuni strains, will be immunogenic during human infection, and will generate a protective immune response. Specific Aim 1. We will identify the protein constituents of the outer membranes of several C. jejuni strains using proteomics and mass spectrometry. Specific Aim 2. We will determine whether OMPs that are conserved in our initial strains are also found in a large number of C. jejuni strains (fresh clinical isolates and an archival collection of strains from across the U.S.), and will evaluate the immune responses of infected humans to these OMPs. Specific Aim 3. We will determine whether immunization of mice with conserved, purified recombinant OMPs protects against subsequent experimental C. jejuni infection.

**Grant:** 1R01AI058810-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** RAMSAY, ALISTAIR J PHD  
**Title:** Vaccination strategies against pulmonary tuberculosis  
**Institution:** LOUISIANA STATE UNIV HSC NEW ORLEANS NEW ORLEANS, LA  
**Project Period:** 2004/04/01-2009/03/31

DESCRIPTION (provided by applicant): Tuberculosis (TB) is the most frequent cause of death from a single infectious agent. Active disease follows about 5% of exposures, but most develop a 'latent' infection without symptoms that may reactivate later, particularly during immune deficiency. Live *M. bovis* bacillus Calmette-Guerin (BCG) is the only currently available vaccine but has had negligible impact on the global epidemic. Indeed, the rise of HIV infection has raised serious safety concerns, with disseminated infection reported in HIV-seropositives following BCG vaccination. Clearly there is an urgent need for safer, more effective vaccination for protective responses that, ideally, will prevent or contain latent TB infection. We have recently shown that consecutive immunization with DNA vaccines and attenuated fowlpoxvirus vectors encoding similar vaccine antigens generates high levels of antigen-specific, interferon-gamma-secreting CD4+ and CD8+ T cells that, importantly, exhibit markedly increased sensitivity (avidity) for the immunizing antigen. In terms of memory, antigen challenge led to rapid expansion of systemic and mucosal T cell effectors in vaccinated animals, reaching levels as high as 30% of total T cell numbers. Mucosal T cells may be particularly important in pulmonary TB, where little attention has been paid to local immune responses. Here, our primary aim is to test the hypothesis that T cell responses induced by prime-boost vaccination will control primary TB infection and the establishment or reactivation of latent infection. Our goal is to generate protective T cell responses against key antigens of *M. tuberculosis* (MTb) normally expressed (i) in acute infection and (ii) during progression to a nonreplicating persistent state. The latter are poorly studied but are of central importance to our proposal, since they represent novel and highly selective targets for vaccine strategies that may forestall or contain latent infection. Specifically, we will test the protective efficacy of systemic prime-boosting against (i) low-dose aerosol challenge with MTb and (ii) reactivation in murine models of latent TB disease. We will also study (iii) the protective capacity of prime-boost vaccines given mucosally and characterize systemic and mucosal (pulmonary) CD4+ and CD8+ T cell responses in these models. Co-delivery of IL-15 genes will be tested for their ability to enhance magnitude and memory of T cell immunity. The capacity of our approach to generate strong, sustained Th1-type CD4+ and CD8+ T cell responses is highly attractive in the context of TB and HIV infection, where maintenance of such responses against key proteins of MTb may be critical for protection.

**Grant:** 1R01AI059048-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** NORRIS, STEVEN J PHD  
**Title:** Virulence Determinants of *Borrelia burgdorferi*  
**Institution:** UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX  
HOUSTON  
**Project Period:** 2004/04/01-2007/03/31

**DESCRIPTION** (provided by applicant): *Borrelia burgdorferi* and related spirochetes are invasive, nontoxigenic pathogens that cause the long-term infection and multiple manifestations associated with Lyme borreliosis. Until recently, genetic manipulations of low passage *B. burgdorferi* with retention of infectivity have not been possible. Presence of the linear plasmids Ip25 and Ip28-1 correlates with infectivity, and recent studies have shown that Ip25 and another linear plasmid, Ip56, greatly reduce the transformation rates of *Borrelia*; rare clones that have spontaneously lost Ip25 (and thus infectivity) are preferentially transformed by shuttle vectors. Preliminary data presented in this application confirms that the large open reading frame BBE02 in Ip25 encodes an enzyme with both restriction and modification activities. A disruption mutant of BBE02 has been isolated that is readily transformable with a shuttle plasmid and retains infectivity. In addition, a recent publication by our group demonstrates that introduction of the Ip25 gene BBE22 (*pncA*) restores infectivity to noninfectious clones lacking Ip25. Taken together, these findings provide the genetic tools required for a systematic analysis of the virulence determinants of *B. burgdorferi*, as described in this proposal. In Specific Aim 1, site-directed mutagenesis will be used to disrupt a number of genes thought to be involved in the mammalian or tick phases of the *Borrelia* life cycle, including *ospAB*, *ospC*, *dbpAB*, *ospEF*, and *visE*. The effects of these mutations on infection of C3H/HeN mice and ticks will be investigated, and the correlation with gene function confirmed by gene complementation studies. The goal of Specific Aim 2 is to identify additional genes that are required in the infectious cycle. In vitro transposon mutagenesis or alternatively signature-tagged mutagenesis will be utilized to randomly disrupt genes in an infectious, highly transformable *B. burgdorferi* B31 clone lacking the Ip25 and Ip56 restriction-modification systems that interfere with transformation. These approaches will, for the first time, permit a systematic, global analysis of virulence determinants of Lyme disease *Borrelia* and will thus provide insight into the mechanisms of pathogenesis of these highly invasive spirochetes.

**Grant:** 1R01AI059062-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** NORGARD, MICHAEL V  
**Title:** The RpoN-RpoS regulatory pathway in *Borrelia burgdorferi*  
**Institution:** UNIVERSITY OF TEXAS SW MED CTR/DALLAS DALLAS, TX  
**Project Period:** 2004/05/01-2009/04/30

DESCRIPTION (provided by applicant): *Borrelia burgdorferi* (Bb), the Lyme disease spirochete, undergoes dramatic adaptive changes as it cycles in nature between its diverse tick and mammalian hosts. However, little is known regarding the genetic regulatory networks that modulate Bb's infectivity and virulence. Recently, we discovered a novel regulatory pathway in Bb, wherein one alternative sigma factor (sigma N, sigmaN, sigma54, RpoN) regulates the expression of another alternative sigma factor (sigma S, sigmaS, sigma38, RpoS) which, in turn, governs the expression of key membrane lipoproteins associated with borrelial virulence. This study proposes to build on this initial observation and investigate, at the genetic and molecular levels, the novel RpoN-RpoS regulatory pathway as it pertains to Bb's infectivity and virulence. In Specific Aim 1, we will identify the activator of the RpoN-RpoS regulatory pathway; studies will focus on examining a role for the "response regulator protein 2" (Rrp2) of Bb that is predicted to be a transcriptional enhancer-binding protein. In Specific Aim 2, we will examine the mechanism by which RpoN controls the expression of RpoS, with emphasis on (i) mapping the rpoS promoter and (ii) assessing the binding of RpoN and its activator (Rrp2) to the rpoS promoter and upstream regions. Studies in Specific Aim 3 will investigate the mechanism by which the RpoN-RpoS pathway regulates the expression of a prototypic differentially regulated lipoprotein, OspC. Emphasis will be placed on analyzing key features of the ospC promoter that engender its control by RpoN via RpoS. In Specific Aim 4, we will examine the influence of the RpoN-RpoS pathway on Bb's ability to infect animals, colonize ticks, be vector-transmitted, and cause disease. Finally, in Specific Aim 5, we will use DNA microarrays to identify more globally those genes of Bb influenced by the RpoN-RpoS regulatory pathway. These combined studies will be instrumental in further characterizing the novel RpoN-RpoS regulatory pathway in Bb and for potentially identifying infection- and/or virulence-associated genes that contribute to many aspects of Bb's complex parasitic strategy.

**Grant:** 1R01AI059111-01  
**Program Director:** PETERS, N KENT  
**Principal Investigator:** FOWLER, VANCE G AB  
**Title:** Virulence determinants in *S. aureus* bacteremia  
**Institution:** DUKE UNIVERSITY DURHAM, NC  
**Project Period:** 2004/03/01-2007/02/28

DESCRIPTION (provided by applicant): Despite intensive research, we simply do not understand why the severity of infections caused by *Staphylococcus aureus* varies so widely. The recent advances in molecular genetics have provided an unparalleled opportunity to understand how *S. aureus* genes and genetic changes contribute to the overall severity of illness. Having used my K23 to create the world's largest collection of well-characterized bacterial isolates from prospectively identified patients with *S. aureus* bacteremia, I now propose to use this resource to evaluate determinants of *S. aureus* virulence. The long-term objectives of this project are to: 1) identify bacterial genes influencing the severity of infection in isolates from a large cohort of patients with *S. aureus* bacteremia, and 2) ultimately use these genes to identify novel interventions for the control of *S. aureus* infections. The overall hypothesis of this investigation is that distinct bacterial virulence determinants influence the severity of *S. aureus* infection. We specifically hypothesize that virulence determinants associated with clinical outcome of *S. aureus* infection segregate into clonal groups, identified by Multilocus Sequence Typing (MLST), and can be localized in the genome by comparative genetic hybridization (CGH). In order to test this hypothesis, we propose to: 1) Define the allelic diversity of 1000 *S. aureus* bloodstream isolates using MLST; 2) Define the genomic diversity of a subset of 200 of these *S. aureus* bloodstream isolates using CGH; and 3) Correlate MLST and DNA microarray results, MLST and clinical outcome, DNA microarray and clinical outcome, and make fully characterized isolates available to the scientific community. The products of this grant will include an increased understanding of genetic diversity in *S. aureus* and the role of this genetic diversity in determining the severity of infections caused by *S. aureus*. The full value of the current proposal also includes the potential future payoff and benefit to the research community as a whole if associations between pathogen genotype and clinical outcome, only possible to identify using such a large and clinically well-characterized collection of isolates, can be defined. This work is critical to furthering the understanding of a crucial medical problem because: 1) *S. aureus* is an emerging pathogen, and 2) interventions to reduce *S. aureus* morbidity require a better knowledge of the bacterial determinants of severity of infection. Understanding the bacterial genetic determinants of disease severity in *S. aureus* infections will advance our understanding of staphylococcal pathogenesis and will enable key advances in protecting the public health from this pathogen.

**Grant:** 1R01AI059114-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** HATFULL, GRAHAM F  
**Title:** Integration and excision by serine-integrases  
**Institution:** UNIVERSITY OF PITTSBURGH AT PITTSBURGH PITTSBURGH, PA  
**Project Period:** 2004/02/15-2009/01/31

DESCRIPTION (provided by applicant): Tuberculosis is the leading cause of death from a single infectious agent, i.e. *Mycobacterium tuberculosis*. Development of genetic tools for understanding mycobacterial biology has been advanced through the study of their viruses, the mycobacteriophages. Several well-characterized mycobacteriophages form lysogens in which the phage DNA is integrated into the host chromosome and these integration systems have proven useful both for constructing site-specific integration-proficient vectors but also for elucidating the mechanism and control of site-specific recombination. Several newly characterized mycobacteriophages integrate and excise their DNA using unusual serine-integrases, which contain a catalytic motif similar to those in transposon resolvases and DNA-invertases. A similar protein is involved in the mobility of the prophage-like element,  $\phi$ Rv1, in *M. tuberculosis*. However, little is known about how this class of enzymes catalyzes integrative recombination between their attP and attB sites, or how this same protein catalyzes recombination between attL and attR for excision. Since attP and attB are different, an interesting biochemical and structural problem arises as to how these serine-integrases choose the correct site pairs for productive recombination. Defined in vitro reactions have been established for both  $\phi$ Rv1 and phage Bxb1 integration and are simple, requiring just the correct DNA partners, purified integrase and a simple buffer. There is no requirement for either DNA supercoiling, additional proteins or high-energy cofactors. An in vitro assay has also been established for  $\phi$ Rv1 excision, which requires a  $\phi$ Rv1-encoded recombination directionality factor (RDF). The mechanism and control of these reactions will be determined through analysis of the requirements at the attachment sites, the nature of the interaction between the integrase and RDF proteins and DNA, and mutational dissection of the integrase proteins. These studies will provide important insights into how phage integration and excision operate and how they can be harnessed as genetical tools for understanding *M. tuberculosis*.

**Grant:** 1R01AI059348-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** KOZEL, THOMAS R  
**Title:** B. anthracis:passive immunization with anticapsular mAb  
**Institution:** UNIVERSITY OF NEVADA RENO RENO, NV  
**Project Period:** 2004/06/01-2007/05/31

DESCRIPTION (provided by applicant): *Bacillus anthracis* is surrounded by an antiphagocytic capsule that is composed of poly-gamma-D-glutamic acid (PGA). Despite an essential role for the capsule in the pathogenesis of anthrax, the immune response to the capsule has received little attention, and the protective role of PGA antibody is unknown. The overall hypothesis for this proposal is that PGA antibodies are protective against anthrax. The goal for the study is to generate a library of PGA monoclonal antibodies (mAbs) and to evaluate the properties of the antibodies in vitro and in vivo. In preliminary studies, a strong isotype-switched (IgG) and affinity-matured antibody response has been generated in mice through immunization with PGA in combination with a potent and novel B-cell adjuvant. Five hybridomas that secrete anti-PGA IgG have been generated in a very short time, and production of additional mAbs having distinct epitope specificities is in progress. Thus, the feasibility for generation of a robust antibody response in mice and for production of IgG mAbs has been demonstrated. The specific aims of the proposal are to i) generate a library of PGA IgG mAbs that represent a spectrum of epitope specificities, ii) evaluate the immunochemistry of mAb binding to soluble and capsular PGA, iii) generate IgG subclass switch variants of PGA mAbs, iv) assess in vitro activities of PGA mAbs that may be predictive of protection, and v) evaluate the role of epitope specificity and antibody isotype in determining protection in murine models of cutaneous and inhalation anthrax. Targeting the capsule for vaccine development or immunotherapy is attractive because anticapsular immunity would not be compromised by antibiotic resistance or engineering of an anthrax toxin that is resistant to toxin-based immunity. The proposed determination of protective efficacy is central to any effort at active or passive immunization that targets the *B. anthracis* capsule and would provide proof of concept for passive immunization as a pre- or post-exposure immunotherapy for anthrax.



**Grant:** 1R01AI059502-01  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** WESSELS, MICHAEL R  
**Title:** Virulence regulation in group B Streptococcus  
**Institution:** CHILDREN'S HOSPITAL (BOSTON) BOSTON, MA  
**Project Period:** 2004/07/01-2009/06/30

DESCRIPTION (provided by applicant): Despite progress in maternal prenatal screening and peripartum antibiotic prophylaxis, group B Streptococcus (*Streptococcus agalactiae* or GBS) infection remains a leading cause of neonatal sepsis and an increasingly important cause of serious infection in elderly and chronically ill adults. Although GBS has the capacity to produce life-threatening infection in susceptible hosts, it usually behaves as a harmless commensal, colonizing the gastrointestinal or genital tract of 30% or more of asymptomatic adults. Adaptation of GBS to its human and animal hosts, and the transition from commensalism to invasive infection, is likely to involve a repertoire of bacterial responses to conditions encountered in various host environments. Preliminary studies have identified a putative two-component histidine kinase-response regulator system in GBS that controls expression of at least two prominent virulence determinants. Inactivation of the response regulator, CsrR, in two independent strain backgrounds resulted in a striking increase in the GBS beta-hemolysin/cytolysin and an equally striking reduction in CAMP factor, a secreted protein that produces synergistic hemolysis with the beta-lysin of *Staphylococcus aureus*. The overall objective of this project is to characterize the potential role of this novel two-component regulatory system in adaptation of GBS to the human host, both as a commensal organism and as an invasive pathogen. This goal will be accomplished through three specific aims: (1) to establish the molecular basis of transcriptional regulation by CsrR/CsrS, a novel multigene regulatory system in GBS; (2) to determine, using DNA microarrays, the effects of the Csr system on global gene regulation; and (3) to characterize the role in pathogenesis of the Csr regulatory system in vivo by studying its effects on adhesion and invasion of human epithelial cells, resistance to host immune effectors, and effects on virulence in animal models of GBS infection. Results of in vitro assays of Csr-regulated gene expression under different environmental conditions together those obtained from cell culture and in vivo infection models will link specific environmental stimuli to the functions of the Csr system in GBS adaptation to the host environment. Together, these studies will broaden our understanding of how virulence gene regulation contributes to GBS pathogenesis.

**Grant:** 1R01AI059529-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** BOCKENSTEDT, LINDA K MD  
**Title:** Genesis & Control of Lyme Disease by Innate Immunity  
**Institution:** YALE UNIVERSITY NEW HAVEN, CT  
**Project Period:** 2004/03/01-2009/02/28

DESCRIPTION (provided by applicant): Lyme disease, due to infection with the spirochete *Borrelia burgdorferi* (Bb), is the most common vector-borne disease in the United States. The disease occurs in stages and is largely due to the host immune response to the spirochete as it adapts to persist in the mammalian host. Innate immunity provides the first line of defense against spirochete invasion and is critical for the induction of protective adaptive immune responses. Members of the Toll-like receptor (TLR) family of pattern recognition molecules allow innate immune cells to recognize and respond to Bb components. Although essential for host defense, innate immunity also gives rise to the immunopathology of Bb-associated disease. This proposal is based on our recent findings that absence of MyD88, an intracellular adaptor molecule required for TLR-induced inflammation, does not eliminate disease in Bb-infected mice, but severely impairs the ability of the host to control infection despite strong humoral immunity. The objectives of this proposal are to 1) determine the mechanisms through which Bb activates innate immune cells in vitro in the absence of MyD88-dependent TLR signaling; 2) define the defect in innate and/or adaptive immune response that leads to uncontrolled pathogen expansion in MyD88-deficient mice; 3) using double knock-out mice, determine the contribution of antibody, Fc receptors and I complement in MyD88-independent disease expression; 4) using RNA interference and conditional mutant mice, define the role of MyD88-dependent TLR responses in the control of Bb in the persistent phase of Bb infection; and 4) using Bb gene arrays, determine gene expression of spirochetes that persist in the antibody-responsive host. The results of these studies will provide new insight into the pathways utilized by innate immunity to recognize and respond to Bb and other extracellular pathogens. Understanding the contribution of these pathways to induction of inflammation may suggest new targets for therapeutic intervention or for enhancing immunity in inflammatory disorders, both infectious and non-infectious in origin. For the field of Lyme disease, defining the key molecules expressed by host-adapted spirochetes will move us one step closer toward understanding how this pathogen persists in an immunologically responsive host.

**Grant:** 1R01AI059647-01  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** DEAN, DEBORAH A MPH  
**Title:** C. trachomatis genomics, strain typing, and evolution  
**Institution:** CHILDREN'S HOSPITAL & RES CTR AT OAKLAND, CA  
OAKLAND  
**Project Period:** 2004/04/01-2009/03/31

DESCRIPTION (provided by applicant): Chlamydia trachomatis (CT) is the leading cause of preventable blindness (trachoma) and sexually transmitted diseases (STD) worldwide. While much has been learned about human chlamydial infections in the last decades, we are still lacking a complete understanding of the pathogenesis of CT diseases, and do not have an appropriate tool for precisely typing CT strains both for molecular epidemiologic and basic research studies. The major outer membrane protein (MOMP) of CT is an immunodominant protein and the primary target for serotyping, and hence strain typing along with the *ompA* gene, which encodes MOMP. Yet, MOMP does not fully reflect the phylogeny of the organism or distinguish strains by biologic or phenotypic properties. Other genes/proteins that may contribute to phylogeny, or these properties include the inter-genic region (IGR), polymorphic membrane proteins (Pmps), cytotoxin genes in the replication termination region (RTR) or "plasticity zone", partial tryptophan operon proteins (TrpB/A), Type III secretion system proteins, chlamydial protease- or proteasome-like activity factor (CPAF), and the porin protein, PorB. Further, the majority of research on CT has used laboratory-adapted strains that may not reflect current clinical isolates that are responsible for the myriad of CT diseases described today. Our goal is to advance the genetic discovery initiated by the CT genome sequences of serovars D and L2 and other genomes of the family Chlamydiaceae by providing genome sequences of six of the 13 remaining reference serovars along with genomes of recent clinical isolates to advance our understanding of CT tissue tropism, virulence, disease pathogenesis, and evolution. Within the context of our Specific Aims, we will develop and make publicly available the data and research tools described: 1) Sequence six genomes of the remaining 13 reference serovars of CT and four genomes of selected recent clinical isolates (see #2), and develop a multi-locus sequence typing (MLST) scheme for global epidemiologic studies; MLST screening of the seven remaining reference serovars and ~500 isolates from CT STD and trachoma populations worldwide will fine-tune the MLST and identify unique clinical isolates for additional genome sequencing; 2) Develop a Chlamydia GeneChip for rapid, robust genotyping of CT based on genome data and MLST findings; and 3) Develop a strain identification database to address specific research questions related to unraveling the association between genetic determinants and tissue tropism, virulence, and disease outcome in addition to the evolution of the organism and how new strain types might evolve over time.

**Grant:** 1R01AI059667-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** ERNST, JOEL D  
**Title:** Type I Interferons in Immunity to Tuberculosis  
**Institution:** NEW YORK UNIVERSITY SCHOOL OF MEDICINE NEW YORK, NY  
**Project Period:** 2004/02/15-2009/01/31

DESCRIPTION (provided by applicant): Mycobacterium tuberculosis (Mtb) causes more adult deaths worldwide than any other bacterium, and many strains are resistant to all first-line drugs. These properties contribute to the potential of Mtb as a major public health problem, as well as a potential agent of bioterrorism. In order to develop more effective means of prevention and control of tuberculosis, it is essential to better understand the mechanisms of protective immunity to Mtb. We have used a murine model to discover an essential role for type I interferons (IFN $\alpha$ ) in control of virulent Mtb in vivo. We found that IFN $\alpha$  contributes to control of Mtb infection, revealed in mice that cannot respond to IFN $\alpha$  or to IFN $\gamma$ : STAT1 $^{-/-}$  and IFN $\alpha$ IFN $\gamma$ STAT1 $^{-/-}$  mice exhibit a more rapid progression of infection and higher bacterial loads than IFN $\gamma$ STAT1 $^{-/-}$  mice. Moreover, we found that IFN $\alpha$  and IFN $\alpha$ -responsive genes are induced by infection with Mtb in vivo. We propose to test three (nonmutually exclusive) hypotheses of the roles of IFN $\alpha$  in control of Mtb. First, we will characterize the microbiologic and pathologic course of Mtb infection in mice that lack the ability to respond to IFN $\alpha$ , IFN $\gamma$ , or both, compared to wild-type mice. We will then test the hypothesis that IFN $\alpha$  contributes to control of Mtb by regulating the same genes, or a subset of genes, regulated by IFN $\gamma$ , using microarray and real-time RT-PCR of RNA from lungs of specific mutant mice. Next, we will test the hypothesis that IFN $\alpha$  regulates priming, differentiation, and trafficking of CD4 $^{+}$  and CD8 $^{+}$  T lymphocytes during Mtb infection. If this indicates that IFN $\alpha$  promotes the development of the adaptive immune response to Mtb, we will determine whether IFN $\alpha$  acts indirectly by promoting the maturation and/or trafficking of dendritic cells. Finally, we will test the hypothesis that IFN $\alpha$  contributes to control of Mtb through natural killer (NK) cells, by characterizing the rate and extent of development, differentiation, and trafficking of NK cells in Mtb-infected mutant mice. If this implies that IFN $\alpha$  regulates NK cells during Mtb infection, we will determine whether Mtb infection of macrophages induces expression of ligands for stimulatory NK cell receptors. The proposed experiments will provide improved understanding of innate and adaptive immunity to Mtb, and may guide development of new means of prevention and treatment of tuberculosis, including drug-resistant tuberculosis.

**Grant:** 1R01AI059673-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** SHANKAR, NATHAN PHD  
**Title:** Role of *E. faecalis* Esp in biofilms and UTI  
**Institution:** UNIVERSITY OF OKLAHOMA HLTH SCIENCES OKLAHOMA CITY, OK  
CTR  
**Project Period:** 2004/07/01-2009/06/30

**DESCRIPTION** (provided by applicant): Nosocomial infections by multiple antibiotic resistant bacteria are especially difficult to cure, pose a significant health risk and place an enormous burden on the economy. A leading cause of such nosocomial infections is the Gram-positive bacterium *Enterococcus faecalis*, which ranks high among the most commonly encountered pathogens infecting the bloodstream, surgical sites and urinary tract. In spite of *E. faecalis* being a leading cause of nosocomial infections, little is known about the bacterial factors involved in promoting persistence of enterococci in the nosocomial environment or at infection sites. We recently identified the first pathogenicity island in *Enterococcus faecalis*. Among the virulence traits that defined this element as a pathogenicity island is a gene encoding the surface protein Esp. Esp is enriched among infection-derived enterococcal isolates and has a unique architecture with multiple tandem repeat motifs, a feature characteristic of many bacterial surface protein adhesins involved in binding to host ligands. In previous studies we have determined that due to the variation in the number of tandem repeat units within the structural esp gene, *E. faecalis* cells may express Esp of varying size and structure at the cell surface. Preliminary studies indicate that alterations in the structure of Esp may influence its role in biofilm formation by enterococci on abiotic surfaces, and in colonization of the urinary bladder during infection. The specific aims of this proposal have been formulated to explore these dual functionalities of Esp. We propose to construct mutants lacking specific modular domains of Esp in an isogenic background, and assess the role of each domain in formation of enterococcal biofilms to determine which elements of Esp structure are essential for biofilm formation by *E. faecalis*. We also propose to construct and screen a library of transposon-insertion mutants to identify and characterize additional gene products that may play a role in the biofilm forming property of *E. faecalis*. Mutants identified in these studies will be evaluated to ascertain if mutations that affect biofilm formation also affect interaction with the host urothelium. Finally, urothelial cell receptors that may be bound by Esp will be identified and the role of the various Esp modular domains in colonizing urothelial surfaces will be determined. The long term goals of the proposed research are to be able to better identify and control infections by pathogenic enterococci and to identify new enterococcal targets for therapeutic intervention.

**Grant:** 1R01AI059702-01  
**Program Director:** JACOBS, GAIL G.  
**Principal Investigator:** HUSSON, ROBERT N MD  
**Title:** Characterization of M. tuberculosis kinases PknA & PknB  
**Institution:** CHILDREN'S HOSPITAL (BOSTON) BOSTON, MA  
**Project Period:** 2004/06/15-2009/05/31

**DESCRIPTION (PROVIDED BY APPLICANT):** Despite efforts to broadly implement effective treatment strategies, the global burden of tuberculosis remains extremely high, and the incidence of multidrug-resistant tuberculosis is increasing in many countries. Insight into mechanisms by which M. tuberculosis adapts to the environment of the host during infection may provide new opportunities for the treatment or prevention of tuberculosis, including multidrug-resistant tuberculosis. A striking finding of the M. tuberculosis genome sequence was the presence of genes encoding several eukaryotic-like serine-threonine kinases. All but two of these appear to be receptor type kinases that likely interact with the extracellular environment and transmit signals by reversible phosphorylation of intracellular substrates, resulting in changes in bacterial physiology. Two of these genes, pknA and pknB are essential for M. tuberculosis viability, and are linked in an operon that also includes orthologues of genes involved in cell wall synthesis and cell shape control. We hypothesize that the proteins encoded by these genes mediate signaling pathways that regulate cell division in mycobacteria. The goal of this research is to characterize the function and signaling pathways of PknA and PknB in M. tuberculosis. To achieve this goal, we propose four specific aims: 1) To complete the characterization of the optimal substrate recognition motif of PknA and PknB, 2) To identify the in vivo phosphorylation targets of PknA and PknB, 3) To investigate the expression and function of PknA and PknB in mycobacterial cell physiology, and 4) To begin to investigate the molecules that interact with the extracellular domains of PknA and PknB.

**Grant:** 1R01AI060025-01  
**Program Director:** WINTER, DAVID B.  
**Principal Investigator:** SILVERMAN, NEAL S BA  
**Title:** Activation of Insect Immunity by Gram-negative Bactria  
**Institution:** UNIV OF MASSACHUSETTS MED SCH WORCHESTER, MA  
WORCESTER  
**Project Period:** 2004/02/15-2009/01/31

**DESCRIPTION** (provided by applicant): The innate immune system is the first line of defense against invading microorganisms. When the innate immune system fails to be activated, lethal infection ensues. Conversely, the inappropriate activation of innate immunity can lead to a variety of illnesses, including sepsis, rheumatoid arthritis and lupus. Insects rely solely on an innate immune response to combat pathogens. The study of innate immunity in *Drosophila*, a genetically tractable organism for which advanced genetic methods are available, allows for the rapid progress in the study of innate immunity in the absence of the confounding influence of the adaptive immune response. The study of *Drosophila* immunity has resulted in important contributions to our understanding of immunity in humans, such as the identification of Toll receptors. The long-term goal of this proposal is to elucidate the molecular mechanisms responsible for the recognition of gram-negative bacteria and the subsequent activation of the signal transduction pathways which culminate in the expression of antimicrobial peptide genes in *Drosophila*. The IMD signal transduction pathway is critical for immune activation and survival following gram-negative infection. The proposed experiments will characterize the gram-negative bacterial products that are recognized by the receptor PGRP-LC, and uncover the mechanisms by which PGRP-LC activates the IMD intracellular signaling pathway. This pathway requires the kinases dTAK1 and *Drosophila* IKK as well as the caspase Dredd, and culminates in the activation, by caspase-mediate cleavage, of the *Drosophila* NF- $\kappa$ B homolog Relish. We propose to investigate the mechanism(s) by which the cleavage of Relish is controlled by IKK-mediated phosphorylation. We will characterize a parallel signal transduction pathway, also activated via PGRP-LC, that involves JNK kinase activation, and results in the expression of a number of novel genes likely to be involved in host defense. We believe that the successful completion of our Aims is likely to lead to a better understanding of the innate immune response, in humans and flies. Such an understanding is critical to our ability to devise more effective therapies against inflammatory disease.

**Grant:** 1R01AI060662-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** DIXON, JACK E PHD CHEMISTRY:CHEMISTRY-UNSPEC  
**Title:** Molecular Mechanism of Pathogenesis  
**Institution:** UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA  
**Project Period:** 2004/05/15-2009/04/30

DESCRIPTION (provided by applicant): A Yersinia effector known as YopT and a Pseudomonas avirulence protein known as AvrPphB define a family of 19 proteins involved in bacterial pathogenesis. We show that both YopT and AvrPphB are cysteine proteases, and their proteolytic activities are dependent upon the invariant C/HID residues conserved in the entire YopT family. YopT cleaves the post-translationally modified Rho GTPases near their carboxyl termini, releasing them from the membrane. This leads to the disruption of actin cytoskeleton in host cells. The proteolytic activity of AvrPphB is essential for autoproteolytic cleavage of an AvrPphB precursor as well as for eliciting the hypersensitive response in plants. The biochemical functions of most avirulence (Avr) proteins are unknown. This proposal focuses on developing a molecular understanding of how the AvrPphB family of proteins is activated and post-translationally modified. In addition, efforts to identify the substrate(s) for AvrPphB are described. Finally, we propose to obtain the x-ray structure of AvrPphB complexed with a peptide substrate. These experiments will provide us with new insights into the molecular mechanism of pathogenesis.



**Grant:** 1R01AI060841-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** SOUSA, MARCELO PHD  
**Title:** Mechanism of Enzymes Mediating Resistance in Pseudomonas  
**Institution:** UNIVERSITY OF COLORADO AT BOULDER BOULDER, CO  
**Project Period:** 2004/07/01-2009/06/30

DESCRIPTION (provided by applicant): Patients with Cystic Fibrosis (CF) develop chronic airway infections with the opportunistic gram-negative bacteria *Pseudomonas aeruginosa*. Airway inflammation and neutrophilic infiltration without bacterial destruction characterize these infections. It has been recently shown that *Pseudomonas* isolated from Cystic Fibrosis patients have specific, virulence-associated modifications in their lipid A structure. These modifications, which include substitutions with palmitate and 4-aminoarabinose, are responsible for resistance to cationic antimicrobial peptides (CAMPs), an important component of innate immunity and Polymyxin, a CAMP antibiotic. The enzymes responsible for the biosynthesis of 4-aminoarabinose-lipid A are clustered in two loci termed PmrE and PmrHFIJKLM. Mutation of any of these genes except pmrM abolishes 4-aminoarabinose addition to lipid A and resistance to CAMPs. ArnA (PmrI) catalyzes the oxidation of UDP-Glucuronic acid to UDP-4-keto-arabinose, an early step in the proposed biosynthesis of 4- aminoarabinose-lipid A. Inhibition of the pathway for 4-aminoarabinose-lipid A biosynthesis would abolish *Pseudomonas aeruginosa* resistance to antimicrobial peptides, therefore greatly enhancing the host immune response against chronic infections with *Pseudomonas*. The specific aims of this proposal are: Specific Aim 1: Determine the three dimensional structure of ArnA. Specific Aim 2: Biochemically characterize the oxidative decarboxylation reaction catalyzed by ArnA. Specific Aim 3: Establish the need for both ArnA activities in [Ara4NH<sub>4</sub><sup>+</sup>]-Lipid-A biosynthesis. Specific Aim 4: Structure determinations of other enzymes in the [Ara4NH<sub>4</sub><sup>v</sup>]-Lipid-A pathway. To improve both the quality of life and the survival age of CF patients it is crucial that new strategies are developed to manage their pulmonary infections. Given the chronic nature of these infections preventing or abolishing resistance is a fundamental problem. This proposal focuses on the characterization of bacterial targets that mediate resistance to CAMPs.

**Grant:** 1R01AI060872-01  
**Program Director:** LAUGHON, BARBARA E.  
**Principal Investigator:** ZAMECNIK, PAUL MD  
**Title:** Oligonucleotides as Tools for Chemotherapy  
**Institution:** MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA  
**Project Period:** 2004/06/15-2007/05/31

DESCRIPTION (provided by applicant): Completion of sequencing of the human and of bacterial genomes has made it possible to apply the principle of oligonucleotide hybridization competition to the inhibition of nefarious genic expression. We shall focus our efforts on three targets: 1) *M. tuberculosis*, with its uniquely constructed mycobacterial outer cell wall; 2) Cystic Fibrosis, in which for our novel oligonucleotide insertion technique we must pin down the extent of insertion. Present estimates, as a result of over thousand sequencings, are that insertion occurs in 10-25 percent of delta508 mRNA molecules; 3) Huntington's Disease, requiring further studies to firm up our finding of inhibition of expression of Huntington protein in tissue cultures from patients with this disease, using derivatives of antisense oligonucleotides.

**Grant:** 1R01AI060892-01  
**Program Director:** SAVARESE, BARBARA M.  
**Principal Investigator:** ROMPALO, ANNE M. MD OTHER AREAS  
**Title:** Douching Prevention Trial in Mother-Daughter Dyads  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 2004/08/15-2009/01/31

DESCRIPTION (provided by applicant): Douching increases the risk of bacterial vaginosis, pelvic inflammatory disease, HIV acquisition and cervical cancer. Given these health risks, why then do women douche? Recently published data from the multi-center Gynecologic Infections Follow-Through (GIFT) Study reported attitudes of 532 women who douched and found that over half had douched for 5 or more years and most initiated the practice on recommendation of female relatives, most commonly mothers, for the reason of hygiene. This study also reported that women who had been advised by a health professional to stop douching were less likely to consider douching healthful and were more likely to have tried to stop. Douching therefore is a risk behavior for significant reproductive health problems that is clearly modifiable. In Baltimore, which ranks among the top 5 cities in the U.S. for gonorrhea, chlamydia and HIV, douching is a common practice. Recent data collected from almost 300 African American women attending the STD clinic found that 70 percent actively douche. We hypothesize that model-based, structured intervention messages on the adverse health consequences associated with douching that are directed to personal determinants of douching practices will be successful in decreasing or stopping vaginal douching when delivered to young women and their mothers. In order to develop an educational intervention targeting adolescents in Baltimore, we propose to perform qualitative interviews and focus group sessions among teens between 14 and 17 years of age, as well as among their mothers or female relatives, to investigate issues and personal and cultural determinants that impact on their choices to practice vaginal douching and douching practices. After developing and pilot testing the intervention, we will recruit 550 mother/teen dyads from our Adolescent Medicine Clinic and randomized them to receive a three-session, one-hour cognitive behavioral intervention mother/teen) or a similar control program on career opportunities. We will use an experimental design for the intervention phase with a pre-intervention, 3-month and 6-month post intervention A-CASI questionnaire. In analysis, we propose to determine the effectiveness of this cognitive-behavioral intervention on douching behaviors among teens; measure beliefs, intention to douche, and perception of mothers' beliefs about douching as an effect of intervention on douching behaviors among teens; and evaluate the effects of change in douching behaviors on bacterial vaginosis and incident gonorrhea, chlamydia and trichomonas infections among teens.

**Grant:** 1R01AI060899-01  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** BLANCHARD, JOHN S  
**Title:** Bacterial N-Acetyltransferases: Resistance to Regulation  
**Institution:** YESHIVA UNIVERSITY BRONX, NY  
**Project Period:** 2004/05/01-2009/04/30

DESCRIPTION (provided by applicant): The long term goals of the present application are to determine the catalytic mechanisms, three-dimensional structures and physiological function of bacterial N-acetyltransferases. As a result of the intensive genome sequencing efforts of the last decade, and modern bioinformatics approaches to the identification of protein superfamilies, some 10,000 members of the GCN5-related N-acetyltransferase (GNAT) family have been identified. In bacteria, these include family members whose function is (1) the acetylation of aminoglycoside antibiotics, (2) the N-terminal acetylation of the ribosomal proteins S5, S18 and L12, and (3) unknown. The specific aims of the current application are organized to address these three classes of bacterial N-acetyltransferases. Of the thousands of encoded bacterial GNAT proteins, only three are known to acetylate proteins. They are encoded by the rimI, rimJ and rimL genes that are presumed to function in the alpha-N-acetylation of their cognate substrates; the ribosomal S5, S18 and L12 proteins, respectively. The state of acetylation of the latter protein is correlated with bacterial growth, suggesting that reversible enzymatic acetylation/deacetylation is important in controlling bacterial growth. Bacterial resistance to antibiotics is a clinically significant problem that threatens current paradigms of antibacterial chemotherapy. Aminoglycosides were one of the first classes of antibiotics used in the treatment of bacterial infections, and act by specifically inhibiting bacterial protein synthesis. Clinically, the vast majority of resistance is due to the expression of enzymes that modify the drug, including enzymes that phosphorylate, adenylate or acetylate aminoglycosides. Of these three activities, the expression of aminoglycoside N-acetyltransferases is most prevalent in clinical isolates. We will continue our examination of bacterial aminoglycoside N-acetyltransferases. Finally, in the genomes of the important human pathogens, *Salmonella typhimurium* and *Mycobacterium tuberculosis*, there are 29 and 20, respectively, predicted GNAT family members, for which only 8 and 4, respectively, have putative, annotated functions, most of which include the functions discussed above. We will develop reagents and methods to define the physiological substrates for these enzymes.

**Grant:** 1R01AI061021-01  
**Program Director:** COYNE, PHILIP  
**Principal Investigator:** WERBOVETZ, KARL A PHD  
**Title:** Simple, Selective Antimitotic Antiparasitic Agents  
**Institution:** OHIO STATE UNIVERSITY COLUMBUS, OH  
**Project Period:** 2004/07/01-2008/06/30

DESCRIPTION (provided by applicant): There is a critical need for the development of cheap and effective drugs against trypanosomiasis and leishmaniasis. Specific inhibitors of parasite tubulin, a known drug target for anticancer and anthelmintic agents, would be of great interest as chemotherapeutic candidates against these diseases. We have thus set out to test the hypothesis that tubulin can be exploited as a target for selective antitrypanosomal and antileishmanial chemotherapy. In support of this hypothesis, our laboratory has identified N1-aryl-N4- dialkyl-3, 5-dinitro sulfanilamides as potent and selective ligands for tubulin from *Leishmania* and trypanosomes. Our best compounds in this series are effective in vitro against *T. brucei* at mid-nanomolar concentrations and against *L. donovani* at low micromolar levels, interfere with parasite cell division at IC50 concentrations, selectively block the in vitro assembly of purified leishmanial tubulin at low micromolar levels, and possess an in vitro selectivity of two orders of magnitude for African trypanosomes over mammalian cell lines. In attempts toward achieving our long-term goal on this project, to develop new antiparasitic drugs that selectively target kinetoplastid tubulin, we propose the following Specific Aims: 1. Synthetic optimization of our simple antimitotic antiparasitic agents by creating a focused library of over 500 N1-aryl-N4-dialkyl-3,5-dinitro sulfanilamides prepared by parallel synthesis. Replacement of the nitro groups will also be investigated through traditional synthetic methods. 2. Evaluation of the target compounds for antiparasitic efficacy, selectivity, and antimitotic activity. Established in vitro protocols will allow us to efficiently select the most promising antimitotic agents against African trypanosomes, American trypanosomes, and *Leishmania*, while animal studies will examine the in vivo antitrypanosomal efficacy and pharmacokinetics of promising compounds. 3. Identification of the binding site of our antimitotic agents through computer modeling and the structure based design of new classes of selective antitubulin agents. These studies will provide insight into the molecular basis for the specificity of our current compounds and will also present an opportunity to identify novel agents with selective antimitotic antiparasitic activity that could serve as drug candidates.

**Grant:** 1R01AI061528-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** MAHMUD, TAIFO PHD  
**Title:** BIOSYNTHESIS APPROACH TO NOVEL BIOACTIVE AMINOCYCLITOLS  
**Institution:** OREGON STATE UNIVERSITY CORVALLIS, OR  
**Project Period:** 2004/06/01-2008/05/31

**DESCRIPTION** (provided by applicant): The increase of multi drug resistance (MDR) among pathogenic bacteria and fungi towards currently used antibiotics coupled with the lack of effective and safe medications to combat various physiological and regulatory disorders such as autoimmune diseases (e.g., multiple sclerosis, amyotrophic lateral sclerosis, etc.) and cancer urgently require new drug discovery. The CTN aminocyclitols, a relatively newly recognized class of microbial secondary metabolites, has great potential to be developed as drugs for various physiological disorders and infectious diseases. This is due to their resemblance to sugar moieties, which are widely involved in various ways in structural and physiological systems in living organisms. In this application, we propose to study the biosynthesis of C7N aminocyclitol-containing natural products and to use the knowledge obtained to develop pharmaceutically important leads via biosynthetic-based structure modifications. The study will be carried out with three different compounds: (1) the antifungal agent validamycin (in *S. hygroscopicus*); (2) the antibiotic pyralomicin (in *Nonomuraea spiralis*); and (3) the anti-tumor cetoniacytone (in *Actinomyces* sp.). The long-term objectives of this study include developing new CTN aminocyclitol-based drugs to combat infectious diseases and physiological disorders, improving production yields and/or providing alternative production strategies of clinically important CTN aminocyclitol compounds, and providing insights about the occurrence and distribution of this class of natural products in nature. The approach employs molecular genetics, enzymology, and chemistry to access, utilize and manipulate CTN aminocyclitol biosynthesis genes that direct precursor formation and other genes involved in the tailoring processes to create novel biologically active compounds. The study includes cloning and elucidation of the biosynthetic gene clusters of validamycin, pyralomicin, and cetoniacytone; elucidation of the newly discovered 2-epi-5-epi-valiolone pathway; characterization of the key biosynthetic enzymes; and use the information obtained to create novel bioactive aminocyclitols. The knowledge and methods that arise from these studies will be directly applicable to expanding the chemical diversity in other families of bioactive natural products.

**Grant:** 1R01AI061577-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** SMILEY, STEPHEN T BS  
**Title:** Cell-mediated protection against pneumonic plague  
**Institution:** TRUDEAU INSTITUTE, INC. SARANAC LAKE, NY  
**Project Period:** 2004/09/30-2008/01/31

DESCRIPTION (provided by applicant): *Yersinia pestis* is the etiologic agent of pneumonic plague. Antibiotic-resistant strains of *Y. pestis* exist, and no licensed vaccine can prevent pneumonic plague. As such, intentionally aerosolized *Y. pestis* has the potential to cause a devastating pandemic. Subunit vaccines comprised of the *Y. pestis* F1 and/or V proteins provide mice and non-human primates with significant, but incomplete, protection against aerosolized plague. Neutralizing antibodies apparently play an important role in that protective response. CD4+T cells presumably contribute to protection as well, since they are critically important for memory B cell responses and the affinity maturation of antibodies. We hypothesize that, in addition to stimulating, maintaining, and/or boosting antibody responses, appropriately primed CD4+T cells can also direct cellular immunity at host cells harboring *Y. pestis* organisms. Relevant prior studies have been limited in scope, although they established that the F1 and V proteins do elicit significant CD4+T cell responses. Here, we propose to (i) determine the immunodominant epitopes recognized by V-specific CD4+T cells in C57BL/6 mice, (ii) develop sensitive assays for quantifying cytokine-producing V-specific T cells, (iii) optimize vaccination protocols to recruit V-specific CD4+T cells to inflamed pulmonary tissues, and (iv) decisively evaluate the capacities of vaccine-primed CD4+T cells to protect against pneumonic plague. Through these studies we will specifically test our hypothesis that vaccine-primed CD4+T cells can combat pneumonic plague via their capacities to stimulate cellular immunity. Subsequent experiments will decipher the precise underlying mechanisms, and strive to develop vaccination protocols that optimally elicit those activities. Acquiring direct information about the activation, expansion and persistence of *Y. pestis*-specific CD4+T cells in lung and lymphoid tissues, and optimizing vaccination strategies to harness the protective capacities of those cells, will undoubtedly aid the development of effective pneumonic plague vaccines.

**Grant:** 1R01AI061598-01  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** MILLER, JEFFERY F  
**Title:** Type III Secretion in Bordetella  
**Institution:** UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA  
**Project Period:** 2004/07/01-2009/06/30

DESCRIPTION (provided by applicant): The Bordetella genus includes several closely related subspecies of Gram-negative bacteria that colonize ciliated respiratory epithelial surfaces in mammals. In addition to their importance as infectious agents, members of the Bordetella genus provide excellent models for probing bacterial-host interactions. The availability of highly related subspecies with different host adaptations allows comparative studies of pathogenesis. Although *B. pertussis* is exclusively adapted to humans, *B. bronchiseptica* has a remarkably broad host range that includes a variety of laboratory animals. *B. bronchiseptica* models allow assessments of the molecular basis of pathogenesis in the context of natural host-parasite interactions. We have identified a type III secretion system (TTSS) in *B. bronchiseptica* that appears to play a key role in facilitating persistent infection of the respiratory epithelium. Recent results indicate that type III secretion (TTS) apparatus genes, regulatory genes, and genes encoding secreted proteins are actively transcribed in *B. pertussis*. Furthermore, the BtrS regulatory system that controls TTS is present and functional. The objective of this proposal is to conduct a comprehensive comparative analysis of TTS in Bordetella subspecies infectious for humans and other animals. Results from our studies will contribute to a fundamental understanding of mechanisms of pathogenesis and the evolution of bacterial virulence. Specifically, we propose to: 1. Conduct a comparative analysis of the BtrS regulons in Bordetella subspecies. BtrS is a newly identified sigma factor that sits at the top of a complex regulatory hierarchy controlling expression of type III secretion loci and other genes. These studies have the potential to discover novel virulence factors and regulatory mechanisms. 2. Determine the roles of "partner switcher" homologs in the regulation of type III secretion. Partner switching represents a new and expanding paradigm in bacterial regulation. We will test the hypothesis that differences in the behavior of the BtrU,V,W partner switching complex accounts for differences in the control of type III secretion between Bordetella subspecies. 3. Investigate the effects of type III secretion and other BtrS-regulated phenotypes during respiratory tract infection by *B. pertussis* and *B. bronchiseptica*.



**Grant:** 1R01AI061712-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** KARIN, MICHAEL PHD  
**Title:** How Anthrax lethal factor kills macrophages  
**Institution:** UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA  
**Project Period:** 2004/06/01-2009/05/31

DESCRIPTION (provided by applicant): Pathogen-induced macrophage apoptosis is an important mechanism used by several highly pathogenic bacteria to avoid detection by the innate immune system through killing of host macrophages, allowing them to establish highly virulent infections. We found that *Bacillus anthracis*, the causative agent of anthrax, can induce macrophage apoptosis and proposed that this process is an important contributor to its mechanism of pathogenicity. Using *B. anthracis* as a model, we found that induction of macrophage apoptosis requires inhibition of anti-apoptotic gene expression and activation of Toll-like receptor 4 (TLR4). Normally, TLR4 engagement results in macrophage activation and cytokine production, as well as induction of genes whose products prevent macrophage apoptosis, but *B. anthracis* uses its lethal toxin (LT) to inhibit activation of p38 MAP kinase (MAPK) and thereby prevents induction of macrophage survival genes. We identified several candidate macrophage survival genes whose induction is p38-dependent and is therefore LT-sensitive. We will continue with characterization of these genes and determination of their physiological role in the maintenance of macrophage survival. We also plan to identify the exact mechanism through which activation of p38 MAPK contributes to induction of these genes in response to TLR4 engagement. We have also identified the major mechanism through which TLR4 engagement can trigger macrophage apoptosis. The critical component of this mechanism is the double stranded (ds) RNA-responsive protein kinase PKR. Importantly, disruption of the gene encoding PKR protects macrophages from the apoptotic effect of *B. anthracis*, *Yersinia* and *Salmonella*. As these results were obtained *ex vivo*, we plan to study in detail the role of PKR in pathogen-induced macrophage apoptosis *in vivo* and determine whether inhibition of PKR can prevent macrophage apoptosis and reduce the burden and lethality of infections with macrophage-killing bacteria, such as *B. anthracis*. Assuming that PKR inhibition may provide a viable strategy for preventing pathogen-induced macrophage apoptosis, we will study in detail the mechanism through which TLR4 engagement leads to PKR activation. Interference with this activation process may be used to increase host resistance to bacterial infections, both with agents of bioterrorism and more common pathogens.

**Grant:** 1R01AI062415-01  
**Program Director:** NEAR, KAREN A.  
**Principal Investigator:** LEE, RICHARD EDWARD PHD CHEMISTRY  
**Title:** Development of new furanyl anti-tuberculosis inhibitors  
**Institution:** UNIVERSITY OF TENNESSEE HEALTH SCI CTR MEMPHIS, TN  
**Project Period:** 2004/05/20-2008/04/30

DESCRIPTION (provided by applicant): Tuberculosis is the leading AIDS associated opportunistic infection found in non-industrialized nations of the world. There is an urgent need to develop new, potent, fast acting anti-tuberculosis drugs that can be used in conjunction with drugs used to treat HIV infections. Towards these ends we propose to explore and develop a novel class of nitrofuranyl amides as new anti-tuberculosis agents. This series of compounds is particularly attractive for TB drug development because of its ease of synthesis allowing for rapid synthesis of analogs in this proposal and could ultimately lead to an inexpensive drug. The goals of this proposal are to enhance the activity of the series in vitro and in vivo and to produce viable preclinical drug candidates. Computational drug design methods and modern medicinal chemistry synthesis techniques will be used to design and synthesize further compound generations. A complete microbiological and biochemical assessment of the nitrofuranyl series will be performed. MIC and MBC determinations for the new generations of compounds will be determined against various *M. tuberculosis* strains including H37Rv and multidrug resistant tuberculosis. The resistance frequency of *M. tuberculosis* to the lead compounds will be examined and cross resistance to other anti-tuberculosis drugs will be investigated. Synergy tests will be performed with other anti-tuberculosis drugs and the cytotoxicity of the compounds determined. The mechanism of action of these inhibitors will be studied. The effectiveness of the leads against latent tuberculosis will be evaluated using an in vitro assay of *M. tuberculosis* grown under low oxygen conditions. Maximum tolerated dose assays and basic bioavailability assays will be performed on the lead compounds. Compounds with good pharmacological profiles will then be tested using a rapid in vivo model. The in vivo activity is then confirmed using the standard TB mouse model. The basic pharmacokinetic and biopharmaceutic properties of leads will be characterized in vivo.

**Grant:** 1R01AI063187-01  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** BENITEZ, JORGE A PHD  
**Title:** Cholera Pathogenesis, mucinase activity and motility  
**Institution:** MOREHOUSE SCHOOL OF MEDICINE ATLANTA, GA  
**Project Period:** 2004/09/01-2008/02/29

DESCRIPTION (provided by applicant): Cholera is a diarrheal disease caused by *Vibrio cholerae* that affects highly populated regions in Asia, Africa, and Latin America. In spite of significant efforts we still lack a vaccine that protects against both serogroups O1 and O139. Several live attenuated *V. cholerae* vaccine candidates still induced mild to moderate diarrhea in clinical trials. The reactogenicity of live vaccine candidates has been correlated with the capacity of attenuated vibrios to penetrate the protective mucus barrier. *V. cholerae* produces a metalloprotease: hemagglutinin/protease (Hap) encoded by *hapA* that has been associated with reactogenicity and promotes mucus penetration and detachment in vitro. We have demonstrated that expression of *hapA* requires the cyclic AMP receptor protein and the stationary phase sigma factor *S*. The aim our study is to determine the mechanism by which quorum sensing regulators and stationary phase factors control expression of *hapA* in responses to environmental stimuli and to characterize the role *hapA* in pathogenesis. To this end we will construct and analyze regulatory mutants at the mRNA, protein and functional level. This study will contribute to a better understanding of the coordination between global regulatory networks in vibrios and other enteric pathogens. Experiments with mutants lacking Hap mucinolytic activity using cell culture, animal models, and volunteers suggest that mucinase production could perturb the protective mucus barrier, promote detachment, and cooperate with motility to spread the infection. In order to prove this hypothesis, we will construct mutants lacking mucinase activity and motility as well as double mutants. We will examine the effect of these mutations on virulence and colonization. Finally, we will use in vivo expression technology to determine the effect of Hap and motility inactivation on the expression of *V. cholerae* main virulence factors: cholera toxin and toxin co-regulated in the infant mouse intestine. This knowledge will facilitate the development of improved cholera vaccines.

**Grant:** 1R01AI063211-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** GIRON, JORGE A PHD  
**Title:** Novel YcbQ Pili of Enterohemorrhagic E. coli O157:H7  
**Institution:** UNIVERSITY OF ARIZONA TUCSON, AZ  
**Project Period:** 2004/09/15-2008/02/28

DESCRIPTION (provided by applicant): Enterohemorrhagic E. coli (EHEC) O157:H7 is recognized as an important emerging pathogen responsible for producing hemorrhagic colitis and the hemolytic uremic syndrome (HUS) in humans. EHEC O157:H7 strains elaborate a potent Shiga toxin, which has been associated with the pathogenesis of HUS. No pili have yet been reproducibly identified in O157:H7 strains and therefore, it is still an enigma as to whether pili play a role in colonization of the intestine of their natural bovine or accidental human hosts. We have recently identified and purified a novel pilus structure produced by EHEC strain EDL933 and other O157:H7 strains. These pili are composed of an 18- kDa pilin subunit and its amino terminus shows identity to the predicted product of the ycbQ gene contained in the EDL933 chromosome. Sequence comparison analysis revealed that these pili, herein called YcbQ, belong to the virulence-associated pili family composed of F17, K99, and G pili found in several animal and human pathogenic E. coli strains, and CupA pili of *Pseudomonas aeruginosa*. Four genes (ycbQ, ycbR, ycbS, ycbT) with homology to F17 family piliation genes were identified by sequence homology in the genome of EDL933, being ycbQ the structural gene. Overall, the objective of this proposal is to advance knowledge of EHEC pathogenesis by elucidating the mechanism(s) of adherence of EHEC O157:H7 to human epithelial cells in culture. Several multidisciplinary approaches involving molecular biology, cell biology, ultrastructural analysis by high power electron microscopy, and biochemical and antigenic analysis will be carried out to extend our current knowledge on the interaction of EHEC O157:H7 with host target cells. The outcome of this proposal will provide important implications for diagnosis of EHEC disease and detection of O157:H7 in food sources and reservoirs. The information obtained will be important for prevention and control strategies of EHEC infections. The central focus of this proposal lies in the following specific aims: 1) To define the genes required for YcbQ pili biogenesis; 2) Define the role of YcbQ pili; and 3) Study transcriptional expression of ycb genes.

**Grant:** 1R01AI063940-01  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** LUKEHART, SHEILA A  
**Title:** Antigenic variation of TprK  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 2004/07/01-2009/06/30

This Program Project application focuses on the paralogous 12-member tpr family of *Treponema pallidum*, the causative agent of syphilis. Evidence suggests that these genes and their encoded proteins are important antigens and virulence factors. We propose to examine TprK, one member of the Tpr family, as the first antigenic variation system to be described in *Treponema pallidum*. TprK is a major target of both cellular and humoral immunity and immunization of rabbits with recombinant TprK results in significant attenuation of lesion development following intradermal challenge with viable *Treponema pallidum*. TprK is heterogeneous among and within strains\* with sequence variation limited to 7 discrete variable (V) regions that are targets of the antibody response. A molecular mechanism has been proposed for the sequence variation, with mutations arising in the single tprK expression site by gene conversion of donor sites located near tprD on the chromosome. Using a novel method for derivation of clonal isolates of *T. pallidum*, we have demonstrated that the tprK V region sequences change during infection, and that variation accumulates under immune pressure. These findings strongly suggest the role of tprK variation in immune evasion and persistence of infection. In this renewal application, we propose the following Specific Aims: 1). Compare the pattern and rate of tprK sequence change among three strains of *T. pallidum* during the course of infection and during serial passage; 2). Determine whether immunosuppression prevents accumulation of variant sequences during infection; 3). Determine whether V region-specific immune pressure selects for organisms with variant tprK sequences; 4). Determine the contribution of TprK V region sequence diversity in susceptibility to heterologous infection or immune escape. These studies, in concert with the aims of Projects 1-3, will help to define mechanisms of pathogenesis and persistence of this important infection.

**Grant:** 1R03AI057760-01  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** SWAMINATHAN, SUBRAMANYAM PHD  
**Title:** Preliminary studies of C. botulinum progenitor toxin B  
**Institution:** BROOKHAVEN SCIENCE ASSOC-BROOKHAVEN UPTON, NY  
LAB  
**Project Period:** 2004/09/30-2006/08/31

**DESCRIPTION** (provided by applicant): Botulinum neurotoxin, released as a protein complex called progenitor toxin by the bacterium *Clostridium botulinum*, causes the disease botulism that is of considerable concern because of its high mortality rate when not treated immediately and properly. It is also a potential biowarfare threat. Its potential use in bioterrorism makes it imperative to develop measures to protect the armed forces and the public in general. The overall objective of this proposal is to aid in developing and designing such measures viz., medicines and vaccines. The long-range goal is to achieve this by understanding the function and purpose of the assembly of the components of C. botulinum progenitor toxins, complex formed by neurotoxin and other associated proteins, by determining the three-dimensional structure of progenitor toxin B via X-ray crystallography. It is believed that progenitor toxins protect the botulinum neurotoxin from the harsh conditions of low pH and peptidases present in the gastrointestinal tract. Knowledge of the three-dimensional organization of these proteins and their interactions with the neurotoxin and one another will lead to the understanding of the mechanism by which they protect the neurotoxin. Progenitor toxin's ability to be absorbed from the gut with minimum degradation makes it a suitable candidate for use as a carrier of oral medicines by appropriate modification of the toxin molecule. Additionally, it will help in designing oral vaccines for many afflictions besides botulism and tetanus. This is particularly important when a large population including animals and birds is to be vaccinated. The short-range goals are to crystallize M and L forms of progenitor toxin B for X-ray diffraction analysis and initiate structural analysis when crystals are obtained. The individual specific aims leading to the overall goal are listed below. 1. To crystallize M and L forms of C. botulinum progenitor toxin B for three-dimensional structure determination via X-ray crystallography. 2. To measure the mass of the progenitor complex with the use of the STEM facility at Brookhaven National Laboratory. 3. To obtain preliminary crystal data from the crystals of M and L forms of progenitor toxin B.

**Grant:** 1R03AI057810-01A1  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** XING, ZHOU PHD  
**Title:** Role of DAP12 in type 1 anti-mycobacterial immunity  
**Institution:** MC MASTER UNIVERSITY HAMILTON, ON L8N 3Z5  
CANADA, ON  
**Project Period:** 2004/07/01-2006/06/30

**DESCRIPTION** (provided by applicant): The mycobacterium is a facultative intracellular pathogen of macrophages and dendritic cells and has proven to be the most difficult microbe for human macrophages/monocytes to kill. The mycobacterium accounts for several infectious diseases of great impact on human health noticeably including tuberculosis (TB) and *M. avium* infection. Incomplete understanding of the mechanisms of protective immune responses to mycobacterial infection has hindered the development of effective vaccines. It is generally believed that type 1 immunity characterized by T cell type 1 cytokine responses and macrophage granuloma formation is essential to host defense against mycobacterial infection. While a number of molecules, both soluble cytokines and cell surface molecules, have been found involved in the initiation and regulation of T cell and macrophage activation during mycobacterial infection, the role of other molecules has remained to be understood. Among these molecules is the recently identified transmembrane protein DAP12. DAP12 is primarily present on macrophages/monocytes, dendritic cells and NK cells and it serves as a signaling molecule for a group of cell surface immunoreceptor associated with DAP12, including TREM-1, TREM-2 and MDL-1. Recently emerging evidence from us and others has suggested that DAP-12-mediated signaling plays an important regulatory role in macrophage activation and inflammatory/immune responses. However, while its role in the development of type I immunity against intracellular bacterial pathogens including mycobacteria has remained completely unknown, our preliminary evidence has strongly implicated DAP12-mediated signaling pathway in host immune responses to mycobacterial infection. Thus, our current R03 proposal sets out to investigate a well-defined, novel question: whether and how DAP12 and its associating molecules are involved in the regulation of type 1 anti-mycobacterial immunity. This proposal carries the following specific AIMS: 1). Investigating the cellular source and regulation of expression of DAP12 and its associating molecules during mycobacterial infection; 2). Investigating the role of DAP12-mediated signaling in type 1 immune responses during mycobacterial infection. We strongly believe that our studies will provide new insights into the mechanisms of type 1 anti-microbial immunity and novel targets for immune modulation.

**Grant:** 1R03AI057826-01  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** BERG, DOUGLAS E PHD  
**Title:** GENETICS OF POLYPHOSPHATE METABOLISM IN H. PYLORI  
**Institution:** WASHINGTON UNIVERSITY ST LOUIS, MO  
**Project Period:** 2003/12/15-2005/11/30

DESCRIPTION (provided by applicant): The control of polyphosphate (polyP) metabolism will be studied in *Helicobacter pylori* (Hp), a fastidious microaerobic pathogen implicated in peptic ulcer disease and gastric cancer. PolyP is a long chain polymer of hundreds of phosphate residues linked by high-energy phosphoanhydride bonds. Studies using fast growing species (e.g., *E. coli*, *P. aeruginosa*; quite unlike Hp in physiology) indicate that polyP is needed for traits such as stress resistance, motility and virulence; and that polyP is made by polyphosphate kinases (PPK), and consumed by PPK or an exopolyphosphatase (PPX). Hp is one of the most genetically diverse of bacterial species. Some of Hp's diversity is postulated to affect important quantitative phenotypic traits that might be seen in studies of metabolic genes. Our initial results indicate that (i) the *ppk1* gene is essential for some Hp strains, but not others; and (ii) in strains for which *ppk1* is dispensable, *ppk1* inactivation can block or impair growth in mice. The proposed studies have two specific aims. First: To better understand roles of *ppk1* and polyP in Hp, and diversity among Hp strains in these roles. Here we will further test the inferred essentiality of *ppk1* in certain Hp strains; select for genes or mutant alleles that at least partially compensate for *ppk1* inactivation; and study the interplay between exopolyphosphatase (PPX) and PPK1. Second: To learn if the need for PPK1 or polyP in vivo depends on host genotype or physiology, and if PPK1 is needed primarily to establish, or also to maintain, infection. These tests will involve experimental infections using appropriate human Hp strains of mouse lines that differ markedly in inflammatory responses (e.g., IL-10 and IL-12 knockout), and also gerbils, a more permissive host. Collectively, the proposed studies will increase understanding of Hp infection mechanisms and associated disease, and perhaps of who to treat for infection and how such treatment can be most effective.



**Grant:** 1R03AI057867-01  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** EATON, KATHRYN A DVM VETERINARY MEDICINE  
**Title:** Recombinant murine IL-10 produced in situ by H. pylori  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2003/12/15-2005/11/30

DESCRIPTION (provided by applicant): H. pylori has been called the most common infectious disease of humans in the world today. Worldwide, between 50-100% of people are infected with H. pylori, but only a minority of those develop clinical signs of disease. Almost 2 decades of research have resulted in a general consensus that host immune response is a critical factor in determining the outcome of infection. Gastritis due to H. pylori is a T helper-1-mediated immune response associated with high levels of IFN $\gamma$  and low levels of IL-10 and other anti-inflammatory mediators. Understanding cytokine function in gastritis due to H. pylori has both diagnostic and therapeutic significance. First, identification of regulatory cytokines such as IL-10 or TGF $\beta$  could lead to further understanding of regulatory pathways and development of therapies for those individuals with intractable infections. Second and perhaps equally important, such understanding could lead to the ability to identify individuals likely to be responders or non-responders. Individuals vary widely in their response to H. pylori, likely because of varying immunoreactivity of each individual host. Because of the strong association between IL-10 and immunoregulation of H. pylori responses, we have chosen to focus this pilot study on IL-10. Our overall goal is to test the hypothesis that recombinant murine IL-10 produced in situ by H. pylori ameliorates or prevents gastritis in mice. This goal will be accomplished in 2 specific aims: Aim 1: To engineer H. pylori to express recombinant murine IL-10. Aim 2: To determine if in vivo expression leads to prevention or resolution of gastritis in response to recombinant bacteria or to wild-type H. pylori that co-colonize with recombinant strains. Briefly, we will construct recombinant H. pylori that expresses murine IL-10, demonstrate that the cytokine is expressed by bacteria in culture and in the mouse stomach, and determine if such expression ameliorates or prevents inflammation in a mouse model of severe gastritis. A successful outcome will not only determine the potential therapeutic role of IL-10 in gastritis due to H. pylori, but will also establish a model in which the roles of other cytokines and mediators can be determined, and host and bacterial interactions can be directly evaluated in vivo.

**Grant:** 1R03AI057915-01  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** LAU, GEE W PHD  
**Title:** Genomics of *P. aeruginosa* Virulence Against Toll  
**Institution:** UNIVERSITY OF CINCINNATI CINCINNATI, OH  
**Project Period:** 2004/01/01-2005/12/31

DESCRIPTION (provided by applicant): The long-term objective of this proposal is to better understand the interactions between the *Pseudomonas aeruginosa* and innate immunity. Microbial pathogenesis is complex, involving numerous components from both the pathogen and the host. In preliminary studies, we have isolated several virulence factors of *P. aeruginosa* that are relevant in mammalian pathogenesis using *Drosophila* model host system. These results suggest that invertebrates could serve as inexpensive hosts to reveal novel mechanisms used by the pathogen to defeat innate immunity. Similarly, numerous studies have demonstrated that the innate immunity pathways of *Drosophila* and mammals have been conserved during the divergent evolution. However, contrary to previous studies, for the first time, our preliminary data indicate that *Drosophila* Toll signaling is important for resistance to infection by *P. aeruginosa*, a Gram-negative bacterium. Our central hypothesis is that Toll mediated immunity is required during antagonistic interactions with *P. aeruginosa*. Taking advantage of the genetics and rapid life cycle of *Drosophila*, we propose one specific aim to: (i) screen for *P. aeruginosa* virulence genes specifically against *Drosophila* Toll signaling. Upon completion of this work, we expect to improve our understanding of the dynamic interplays between Toll mediated innate immunity and *P. aeruginosa* virulence.

**Grant:** 1R03AI057920-01  
**Program Director:** FALLOON, JUDITH  
**Principal Investigator:** GEISLER, WILLIAM M MD  
**Title:** Immune Responses in Genital Chlamydia Prior to Therapy  
**Institution:** UNIVERSITY OF ALABAMA AT BIRMINGHAM BIRMINGHAM, AL  
**Project Period:** 2004/01/01-2005/12/31

DESCRIPTION (provided by applicant): Despite improved preventive efforts, genital Chlamydia trachomatis infection remains the most common bacterial STD in the United States and is a global public health concern. If untreated, Chlamydia may persist allowing for transmission to uninfected individuals and possible clinical progression to complications. Recent reports studying chlamydia-infected patients between the time of routine testing (screening) and the time they returned for therapy demonstrated evidence of spontaneous clearance of a portion of untreated infections, suggesting immune-mediated clearance of chlamydia. Other than limited observations such as these, most of our knowledge on the host response to C. trachomatis has been derived from animal models and in vitro studies, which have demonstrated the importance of both cell-mediated and humoral-mediated immune responses to C. trachomatis. To assess the role of cell-mediated and humoral-mediated immune responses in clearance of genital chlamydia in humans, we propose a prospective pilot study that will enroll STD clinic patients with positive chlamydia screening tests that return for therapy. Study aims are: (1) determine the percentage of patients who spontaneously clear chlamydia versus having persisting infection by culture upon return for therapy and the relationship of patient age to this outcome; (2) measure proinflammatory and anti-inflammatory cytokines and cytokine gene expression in genital specimens from both patient groups by ELISA, cDNA Gene Array, or RT-PCR; and (3) measure anti-C. trachomatis antibody subtypes in the serum and genital secretions in both groups by ELISA. The relationship of specific immune responses to outcome in chlamydia (resolution versus persisting infection) will be analyzed through parametric or nonparametric methods and multivariate analysis. Study findings will provide preliminary data for further NIH grant applications, which if granted will allow continued enrollment of patients for this prospective study (increasing the sample size and power to detect outcomes) and the ability to test for additional host immune parameters. Findings from this research may improve our understanding of the host pathogen interactions in genital chlamydia and may provide the basis for future efforts towards prevention and control of genital chlamydia in development of novel immune interventions or a vaccine.

**Grant:** 1R03AI058141-01A1  
**Program Director:** GIOVANNI, MARIA Y.  
**Principal Investigator:** SCHWEIZER, HERBERT P. PHD  
**Title:** Genetic Tools for Pathogenic Bacteria  
**Institution:** COLORADO STATE UNIVERSITY-FORT FORT COLLINS, CO  
COLLINS  
**Project Period:** 2004/07/02-2006/06/30

DESCRIPTION (provided by applicant): Bacterial gene analysis has been revolutionized in recent years with the advent of high-throughput technologies that allow sequencing of entire genomes within a matter of months and comparative gene expression analyses of entire genomes within a couple of days. However, the genetic methods required to analyze specific genes identified by some of these high-throughput technologies in defined environments are by comparison slow, outdated and in many instances not even existing outside the better characterized species such as *Escherichia coli*. Furthermore, existing technologies, such as plasmid-based systems are not applicable in certain environments, for example animals and biofilms. In this proposal we seek funds to extend our laboratory's very successful program of genetic tool development by exploring the hypothesis that it is feasible to exploit the molecular properties of the site-specific transposon Tn7 to generate a kit of versatile, broad-host-range, site-specific gene integration vectors that can be used for diverse applications in different pathogenic bacteria. To test this hypothesis and to attain our goal we propose two specific aims. In aim 1 we will improve our prototype mini-Tn7 vectors by insertion of genetic transfer functions and transcriptional terminators, equip them with excisable antibiotic selection markers and reporter genes, and functionally analyze them in the genetically tractable model host *Pseudomonas aeruginosa*. In aim 2, the range of mini-Tn7 vectors will be extended to two other clinically significant respiratory pathogens, *Burkholderia cenocepacia* and *Stenotrophomonas maltophilia*, which are genetically less tractable and chromosomally more complex. The chromosomal insertion sites will be determined and chromosomally integrated lacZ fusions will be constructed and analyzed as proof-of-principle for the applicability of reporter gene analysis from single-copy, chromosomally located reporter genes in these bacteria.

**Grant:** 1R03AI058241-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** MCALPINE, SHELLI R PHD  
**Title:** Novel Antibiotics: Site-Specific Recombination Inhibitor  
**Institution:** SAN DIEGO STATE UNIVERSITY SAN DIEGO, CA  
**Project Period:** 2003/12/01-2005/11/30

**DESCRIPTION** (provided by applicant): The goal of this project is the synthesis of macrocyclic peptides that trap Holliday Junctions (HJ) in bacteria, and their subsequent evaluation as potential antibiotics. The HJ is an intermediate during site-specific recombination, a DNA repair mechanism. Trapping the HJ shuts down DNA repair in bacteria. Because the HJ would be a unique therapeutic target, compounds that trap this intermediate may effectively kill resistant strains of bacteria. This mechanism is reminiscent of the quinolone/fluoroquinolone class of antibiotics, which stabilize a normally transient intermediate, thereby killing bacteria. Outlined are two specific aims designed to accomplish our goal. The first specific aim describes the synthesis of a new class of compounds, C-2 symmetrical macrocyclic peptides, which were designed from lead structures. These lead structures are linear dodecapeptides that exhibit potent bactericidal activity by trapping the HJ. 1,2 The second specific aim (SA2) describes the evaluation of these compounds as potential antibiotics using bacterial growth assays and biochemical assays. Preliminary data demonstrates the success of the proposed strategy, where our macrocyclic compounds trap the HJ in the biochemical assays and demonstrate some antibiotic activity in cell growth assays. Care has been taken to ensure the goals can be realistically accomplished in the two-year time frame for this proposal. These pilot studies are intended to demonstrate the feasibility of this project. Once the viability of the proposed idea has been clearly established, we will submit a more in-depth and expanded proposal.

**Grant:** 1R03AI059117-01

**Program Director:** BAKER, PHILLIP J.

**Principal Investigator:** HALDENWANG, WILLIAM G PHD  
MICROBIOLOGY:MICROBIAL  
BIOCHEMISTRY

**Title:** Optimization of Mariner Transposon for Bacillus

**Institution:** UNIVERSITY OF TEXAS HLTH SCI CTR SAN SAN ANTONIO, TX  
ANT

**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (provided by applicant): Transposable elements are among the most useful tools available for genetic analysis in bacteria. Not only can transposons disrupt operons or tag interesting alleles with selectable markers, but specialized versions of these elements, carrying origins of replication or reporter genes, can be used to directly clone or analyze the expression of the operons that they disrupt. Studies of Gram-positive bacteria, including *Bacillus anthracis* have benefited from a transposon system (Tn917) developed in *B. subtilis*. Although the Tn917 system has been successfully used in a number of instances, it is not without its limitations. Most notably, 99% of all Tn917 transpositions in *B. subtilis* target "hot spots" on the *B. subtilis* chromosome. Recently, the eukaryotic transposable element Himar1 has been modified for use in a number of bacterial systems, where random transposition occurs into a dinucleotide sequence (TA). The current application seeks to develop Himar1 for use in *Bacillus* species. Antibiotic resistance cassettes, selectable in *Bacillus* will be bracketed by the transposon's inverted terminal repeats (ITR) and placed on a plasmid with a temperature-sensitive *Bacillus* origin of replication. The plasmid will also include a hyperactive variant of the Himar1 transposase gene with expression elements appropriate for *Bacillus*. The system will be evaluated in the more genetically tractable *B. subtilis*, and once optimized, embellished with additional elements (e.g., a reporter gene system, origin of replication for cloning) and tested for effectiveness in *B. anthracis*. During development, Himar1 transposase variants, hyperactive in *Bacillus* will be sought using a modification of a papillation assay developed for use in *Escherichia coli* and a sporulation selection protocol unique to *Bacillus*. The transposons created in this study should be of use both in *Bacillus* species and Gram-positive bacteria in general. Additionally, the hyperactive Himar1 transposase variants isolated in this study could be incorporated into existing Himar1 systems to enhance their efficiency, and provide insights into elements of the transposase that are important for its activity or stability.

**Grant:** 1R03AI059234-01  
**Program Director:** ZOU, LANLING  
**Principal Investigator:** NATARAJAN, GITA PHD  
**Title:** NASAL ANTHRAX VACCINE DELIVERY FORMULATIONS  
**Institution:** SRI INTERNATIONAL MENLO PARK, CA  
**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (provided by applicant): The 2001 anthrax emergency in the United States has prompted public health authorities to consider how they might conduct mass immunization campaigns if confronted by a bioterrorism event. The anthrax vaccine now used in the United States requires six subcutaneous injections over an 18-month period, plus yearly boosters. We propose to develop a new, nasally administered, extended-release anthrax vaccine for use as primary or booster immunizations. Our nasal formulation for anthrax antigen will have several important advantages: it will (1) allow easy, non-invasive administration; (2) improve the immune response and reduce the number of shots; (3) be bioadhesive, with a longer residence time because the formulation will be able to adhere to the posterior portion of the nose, where mucociliary activity results in faster clearance of medications and thus increases the bioavailability of the vaccine; (4) increase antigen delivery, enhance antigen stability, and act as an antigen reservoir or depot; and (5) eliminate the need for alum as an adjuvant. The proposed formulation will be a polymer-based, two-part formulation that will be mixed in a hand-held delivery device just prior to administration. (The delivery device is beyond the scope of this project, although such devices have already been used for other vaccines). The study will involve collaborative studies by investigators with expertise in pharmaceutical formulation, vaccine biology, and immunology. The long-term goal is a fast-acting, easily administered, extended-release anthrax vaccine.

**Grant:** 1R03AI059404-01  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** XIA, MINSHENG PHD  
**Title:** Genotypically Matched Chlamydial Strains & IncA Function  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 2004/05/01-2006/04/30

DESCRIPTION (provided by applicant): Chlamydiae are important human pathogens causing a variety of ocular, respiratory and genitourinary infections, and potentially atherosclerotic cardiovascular disease. Particularly Chlamydia trachomatis is a pathogen of tremendous public health importance causing the most common bacterial sexually transmitted diseases, preventable blindness and increased risk for HIV transmission. Despite the clinical and public health importance of this organism, the mechanisms that the organism uses to interact with host cells are not understood. C. trachomatis is an obligate intracellular bacterium, developing and multiplying exclusively within the host cell in a membrane bound vacuole termed an inclusion. Research on chlamydial gene function has been hampered by the lack of laboratory methods for manipulating its genome. We propose an alternative strategy for studying chlamydial inclusion membrane proteins utilizing naturally occurring mutants and their genotypically matched wild type counterpart strains. Because the chlamydial inclusion membrane serves as the interface between the organism and the host cell, inclusion membrane proteins are therefore believed to be essential to chlamydial survival and organism-host interactions. The overall goal of this study is to utilize mutant-wild type strain pairs to assess the role of C. trachomatis IncA in interacting with the host cells. Specifically, in Aim 1 we will identify genetically closely related strains to match the naturally occurring incA mutants as an alternative to laboratory derived mutant-wild type pairs. In Aim 2, we will utilize these mutant-wild type pairs to examine the impact of IncA on host cell gene expression, especially with respect to signal transduction constituents. Results from these studies will provide new understanding of chlamydial inclusion membrane proteins and their interaction with host cells. A better understanding of chlamydial inclusion membrane proteins will provide new insights into chlamydial biology and pathogenesis.



**Grant:** 1R03AI059468-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** ZUECKERT, WOLFRAM R PHD  
**Title:** Structure-Function of Borrelia Surface Lipoproteins  
**Institution:** UNIVERSITY OF KANSAS MEDICAL CENTER KANSAS CITY, KS  
**Project Period:** 2004/04/15-2006/03/31

DESCRIPTION (provided by applicant): Borrelia spirochetes causing relapsing fever (RF) can persist in an infected host by antigenic variation of Vsps and Vlps, two families of variable, immunodominant and thus serotype-defining surface lipoproteins. Vsps and Vlps induce a T-independent neutralizing IgM response, which leads to complement-independent elimination of that specific serotype. Yet, these antibodies do not protect from subsequent spirochetemia of other serotypes. Highlighting an additional evasion strategy, one of two Vsps with different binding affinities to glycosaminoglycans (GAGs) has been implicated in targeting RF Borrelia to the central nervous system, an immunoprivileged site. The overall objective of this proposal is to gain an in-depth understanding of these pathogenic processes by defining the structural and functional basis of Vsp- and Vlp-mediated immune evasion and tissue tropism. Their demonstrated overlapping role in both mechanisms lead us to initially focus on the Vsps. Our preliminary data indicate that Vsps share a conserved dimeric and alpha-helical structural fold, with hypervariable loops connecting the alpha helices. We hypothesize that these variable and membrane-distal loops (i) form the Vsp-specific antibody epitopes, and (ii) also confer the surface properties involved in tissue localization. To significantly facilitate our studies, we have developed a novel surface display system, which allows us to stably express and present functional Vsps and Vlps on the surface of the Lyme disease (LD) spirochete Borrelia burgdorferi. We will now apply this system to test our hypothesis and have formulated two major specific aims: 1. to define the neutralizing antibody epitopes of Vsp proteins: Based on the three-dimensional structure of two Vsps and using specific IgG and newly generated IgM monoclonal antibodies, we will map antibody epitopes by analyzing Vsp escape mutants and antibody interactions with Vsp chimeras and point mutants. 2. to define the Vsp domains interacting with GAGs: Chimeras and point mutants of two Vsps will be examined for binding to purified GAGs, glial and endothelial cells as well as for neurotropic characteristics. These studies will significantly increase our understanding of the pathogenesis of Borrelia infections, and shed more light on the mechanisms of antigenic variation and immune evasion of microbes in general. Furthermore, they will yield important clues for the design of future intervention strategies.

**Grant:** 1R03AI059500-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** WANG, JULIA Y PHD  
**Title:** Chemical Structure of Anthrax Spore Polysaccharide  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 2004/09/01-2006/08/31

DESCRIPTION (provided by applicant): The goal of our study is to determine the chemical structure of the outermost surface component of anthrax spores, exosporium polysaccharide (EPS). Spores of *Bacillus anthracis* are the causative agent of the highly lethal anthrax infection. The anthrax spore consists of several distinct structural layers, which protect the core genetic material. Because of their unique structural properties, anthrax spores are long-lived and extremely resistant to adverse environments. Once inside the host, the same structures allow anthrax spores to survive the host immune surveillance and to germinate, leading to rapidly replicating bacilli and production of toxins that kill the host. Despite these facts, we know surprisingly little about the basic chemical structures of anthrax spore constituents and their functions. EPS is expressed on the surface of anthrax spores, forming a hair-like cover of the spore. EPS remains intact throughout the entire germination stage from dormant spores to newly transformed vegetative bacteria. Because polysaccharides are typically highly stable biopolymers of poor immunogenicity, we hypothesize that EPS plays significant roles in protecting the survival of spores and in shielding spores from immune defense. The elucidation of the EPS structure will provide vital fundamental understanding of anthrax spore biology and more generally of sporulating microorganisms. The EPS structure will reveal useful antigens for the development of methods for rapid detection and efficient destruction of anthrax spores and/or unprecedented spore-directed anthrax vaccine and therapy. Aim 1. Isolation of EPS from *B. anthracis* spores. Spores will be prepared from attenuated, avirulent strains of *B. anthracis*. The exosporium layer will be extracted from the spores. EPS will be cleaved from exosporium proteins by biochemical and enzymatic methods. EPS will be purified by gel filtration and/or ion exchange chromatography. Aim 2. Elucidation of the chemical structure of EPS. A combination of multidimensional high-resolution NMR spectroscopy, mass spectrometry, biochemical or enzymatic degradation, chemical derivatization, and gas chromatography or HPLC will be employed in parallel to elucidate the chemical structure of EPS. If EPS is found to be heterogeneous, we will attempt to characterize the structures of all distinct entities.

**Grant:** 1R03AI059644-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** VISSA, VARALAKSHMI D PHD  
**Title:** Molecular typing of Mycobacterium leprae  
**Institution:** COLORADO STATE UNIVERSITY-FORT COLLINS FORT COLLINS, CO  
**Project Period:** 2004/05/15-2006/04/30

**DESCRIPTION (PROVIDED BY APPLICANT):** An objective of the 1991 World Health Assembly was the elimination of leprosy as a public health problem by the year 2000. The goal was to achieve a reduction in prevalence of leprosy to less than 1 per 10,000 population. This goal has not been achieved in the most endemic countries, where more than 80% of the global leprosy cases occur, despite the implementation of the Multi Drug Therapy (MDT) program. The number of new cases detected has also not reduced indicating that the transmission of the causative agent Mycobacterium leprae has not been controlled. There are fundamental gaps in the understanding of transmission, and a lack of sensitive methods to track leprosy. Questions remain regarding the nature of the reservoir of M. leprae, the route of infection, and mode of its spread. To address these concerns, a DNA typing method for M. leprae is proposed based on the availability of the genome sequence and the principle of multiple locus variable-number of tandem repeat analysis (MLVA), which has been developed for several other infectious organisms. M. leprae cannot be cultivated in a laboratory and bacteria for research applications is mainly derived from susceptible armadillo or nude mice. This pilot study is therefore designed with two specific aims: 1. Development of a standard molecular typing method using a panel of armadillo derived M. leprae isolates and 2. Application of the optimized methods in a panel of M. leprae DNA derived from biopsies and nasal swabs from patients. The methods involve the PCR amplification of multiple genomic loci containing short tandem repeats for each isolate of M. leprae and the comparison of the fragment length of each locus with that obtained from other isolates. Automated electrophoresis, data collection and analysis will be used. The long-term goal of the study is to provide the tools and techniques suitable for future epidemiological studies such as the role of human, non-human and environmental sources of M. leprae in the transmission of leprosy via nasal or dermal routes of entry and release of the pathogen and the differentiation of relapse from reinfection in patients.

**Grant:** 1R03AI059670-01  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** BURNS, JANE L  
**Title:** Effects of azithromycin on *P. aeruginosa* gene expression  
**Institution:** CHILDREN'S HOSPITAL AND REG MEDICAL SEATTLE, WA  
CTR  
**Project Period:** 2004/05/01-2006/04/30

DESCRIPTION (provided by applicant): Cystic fibrosis (CF) is a genetic disease characterized by airway infection and inflammation with early death resulting from chronic airway disease. *Pseudomonas aeruginosa* is the most important pathogen in CF airway infections and anti-pseudomonal antibiotics have long demonstrated efficacy in decreasing morbidity and mortality in CF. However, other antibiotics--such as azithromycin--that do not have classical in vitro antimicrobial activity against *P. aeruginosa* have demonstrated clinical efficacy. A recent clinical trial of azithromycin in the US (for which my laboratory performed quantitative microbiology) demonstrated improvement in lung function and decreased rate of pulmonary exacerbation in those patients who received azithromycin (N = 87) compared with those who received placebo (N = 98), in the absence of a quantitative anti-pseudomonal effect. The mechanism of this activity is not understood, but may be caused by host effects, antibacterial effects or a combination of the two. I have subsequently examined study isolates for an antibiofilm effect and demonstrated no correlation with clinical response. Thus, I hypothesize that azithromycin affects gene expression in *P. aeruginosa*, which may result in decreased inflammation and improved lung function. Three aims are proposed to test that hypothesis. The first of these is to use gene expression arrays to identify genes in strain PAO1 that are regulated by growth in sub-MIC concentrations of azithromycin. Subsequently, the patient isolates from the US trial of azithromycin in CF will be used to determine whether clinical response to the drug correlates with the presence of azithromycin-regulated genes. Finally, differences in gene regulation between those isolates from responders and non-responders will be examined. The data will be analyzed to determine whether there are specific azithromycin-regulated genes whose expression, or lack of it, is correlated with the CF response to azithromycin therapy. It is hoped that elucidation of this mechanism of action may result in other novel therapies. Additionally, specific markers may be identified to predict CF patient response to azithromycin, thus preventing ongoing treatment with ineffective therapy.

**Grant:** 1R03AI060863-01  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** CARBONETTI, NICHOLAS H. PHD  
**Title:** Effect of Pertussis Toxin on Cough in Guinea Pig  
**Institution:** UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD  
SCHOOL  
**Project Period:** 2004/08/01-2006/07/31

**DESCRIPTION** (provided by applicant): *Bordetella pertussis* is a gram-negative bacterial pathogen that causes the disease known as pertussis or whooping cough. After respiratory tract infection by the bacteria, the disease is characterized by a severe and prolonged cough, with frequent paroxysmal coughing episodes at the height of the symptoms that then reoccur with reduced frequency over several weeks or months. Although *B. pertussis* is a human-specific pathogen in nature, small laboratory animal models, such as mice and rats, have been used to study aspects of *B. pertussis* respiratory tract infection and disease, the role of various virulence factors, and the immune responses elicited to this infection. However, a representative and convenient model for the cough symptoms associated with pertussis disease in humans has not been developed. Guinea pigs represent an attractive model for mechanistic studies on cough, because many aspects of their cough pathophysiology, including the stimuli that elicit cough and airway hyperresponsiveness, and the pharmacology of the airway responses, are very similar to those of human cough. However, guinea pigs have not previously been used to study *B. pertussis* infection of the respiratory tract. In the study outlined in this proposal, we aim to test the feasibility of using guinea pigs as a small animal model for aspects of the cough disease associated with respiratory tract infection by *B. pertussis*. In particular, we will address the hypothesis that pertussis toxin, one of the major virulence factors produced by *B. pertussis*, plays a major role in eliciting or modifying the cough symptomology. Our specific aims are: 1. To elucidate the characteristics of *B. pertussis* colonization of the guinea pig respiratory tract, including that of wild type and pertussis toxin-deficient strains of *B. pertussis*; and 2. To determine whether *B. pertussis* infection of the guinea pig respiratory tract either elicits a cough response itself, or modifies the cough pathophysiology induced by commonly used tussive stimuli, and the role that pertussis toxin plays in these effects.

**Grant:** 1R03AI061308-01  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** ADEGBOLA, RICHARD A PHD  
**Title:** Genotypes of Helicobacter Pylori in West Africa  
**Institution:** MRC LABS--GAMBIA BANJUL, GAMBIA WEST AFRICA,  
**Project Period:** 2004/09/01-2006/08/31

DESCRIPTION (provided by applicant): Helicobacter pylori infects over half of adults worldwide. Colonization begins in childhood, and contributes to gastroduodenal disease in adult life. Strains obtained close to colonization are rarely encountered, as they require isolation from young children, yet a detailed study of such strains is critical to understanding H. pylori associated disease. Our study will be conducted in The Gambia, W. Africa, the ancestral home of many African Americans, in a population with a high early childhood incidence of H. pylori colonization. The sadly frequent need for the use of naso-gastric tubes to intensively re-feed malnourished infants, and the high early childhood incidence of H. pylori infection, allow us a unique opportunity to investigate colonizing strains of H. pylori, and further develop appropriate local approaches to disease management. Our goal is to evaluate new methods for culturing H. pylori strains from young children, and to compare these to those obtained from adults. We propose that the transition from childhood colonization to adult disease state is affected by multiple factors including evolution of genotypes of colonizing H. pylori strains themselves, so that H. pylori strains from Gambian children will differ genetically from strains from chronically colonized adults. We have two specific aims: Aim 1: To compare the range of genotypes obtained from H. pylori cultured from gastric biopsies from Gambians, with those obtained by culture of gastric juice aspirate, from the same subjects: Aim 2: To assess the relatedness of child strains and those from close adult relatives, cultured from naso-gastric aspirates. The data from these experiments will give us our first insight into the changes that may occur in the H. pylori genome during the period of colonization of its unique niche, the human stomach. We anticipate that our work will allow further understanding of why the clinical diseases associated with chronic H. pylori colonization in West Africa differ from those seen in industrialized nations, and enable the development of appropriate treatment strategies for communities such as The Gambia.

**Grant:** 1R03AI061526-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** FORMAN, BARRY M. BA  
**Title:** Bacterial AHL interactions with nuclear receptors  
**Institution:** CITY OF HOPE/BECKMAN RESEARCH DUARTE, CA  
INSTITUTE  
**Project Period:** 2004/07/01-2006/06/30

DESCRIPTION (provided by applicant): Free-living bacteria tend to associate with cell or inert surfaces to form surface-associated communities called biofilms. Gram-negative bacteria release small molecules called acyl homoserine lactones (AHLs) into the surrounding environment to communicate within and between species. When sufficient bacteria are present to form a "quorum," the AHLs reach high enough concentrations to trigger developmental programs that change the morphology and biochemical functionality of the cells to adapt them to life in a biofilm community. Among the changes incurred by this so-called quorum sensing system are loss of motility and gain of antibiotic resistance. Biofilms associated with chronic lung infections in cystic fibrosis and septic infections related to medical device implantation are difficult to eradicate with antibiotic therapy due to induction of resistance genes in the biofilm members. Persistent biofilms or bacterial overgrowths that are difficult to eradicate may produce large quantities of these AHLs. Recent evidence suggests that AHLs interact with eukaryotic cell signaling systems and can alter host gene expression. Preliminary molecular modeling suggests that AHLs may be able to bind to the human peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ), a nuclear receptor with important roles in inflammation, obesity, diabetes, and cardiovascular disease that binds a number of small molecule ligands. There is no data either in vitro or in vivo demonstrating whether AHLs in fact bind to eukaryotic nuclear receptors and alter gene transcription. We propose to determine whether bacterially derived AHLs can regulate eukaryotic gene transcription by activating host nuclear receptors including the PPARs, LXRs, FXR, CAR, PXR/SXR, ERs, TRs, and VDR.

**Grant:** 1R03AI061722-01  
**Program Director:** ZOU, LANLING  
**Principal Investigator:** HYBERTSON, BROOKS M PHD  
**Title:** Respirable ciprofloxacin aerosol for inhaled anthrax  
**Institution:** UNIVERSITY OF COLORADO DENVER/HSC DENVER, CO  
AURORA  
**Project Period:** 2004/09/30-2006/08/31

DESCRIPTION (provided by applicant): Pulmonary delivery of solid ciprofloxacin aerosol particles is an attractive possibility for chasing anthrax spores into the thoracic lymph nodes and killing the bacteria when the spores germinate. The overall goal of this project is to develop a method in which inhaled micron-sized particles of ciprofloxacin can be used to benefit victims of inhalational anthrax. We hypothesize that aerosol administered particles of Upophilic ciprofloxacin will be taken up by alveolar macrophages and transported to the thoracic lymph nodes where inhaled anthrax spores vegetate into active bacteria. Our strategy is intended to put the antibiotic at high and sustained concentrations within alveolar macrophages in lung and lymph locations. We anticipate that as the ciprofloxacin particles dissolve, the drug may be released within alveolar macrophages that contain or are near to other cells that contain spores, and be able to kill the bacteria when they germinate. Although several water-soluble antibiotics, including tobramycin, have been formulated for pulmonary delivery as inhaled aerosols, respirable particles of highly lipophilic antibiotics like ciprofloxacin have not, although others are examining the formation of liposomal ciprofloxacin. We have developed an effective method for aerosol delivery of lipophilic drugs based on the use of supercritical carbon dioxide as solvent and propellant. Our goals for this pilot project are to determine whether supercritical fluid drug aerosolization will allow inhalation delivery of ciprofloxacin to the lungs, to determine the uptake and dissolution properties of ciprofloxacin particles in cultured alveolar macrophages, to investigate ciprofloxacin particle translocation to the thoracic lymph nodes, and to determine the pharmacokinetic parameters. Our overall goal is to create a new tool for targeted treatment or prophylaxis for victims of anthrax spore inhalation.



**Grant:** 2R15AI047802-02  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** MELLIES, JAY L PHD  
**Title:** Ler-Mediated Regulation of EPEC Virulence Genes  
**Institution:** REED COLLEGE PORTLAND, OR  
**Project Period:** 2000/09/01-2007/06/30

DESCRIPTION (provided by applicant): Enteropathogenic *E. coli* (EPEC) is a significant cause of infant diarrhea in developing countries. The hallmark of EPEC infection is their ability to cause attaching and effacing (AE) intestinal lesions, and all genes necessary for this phenotype are found within a 35.6 kb pathogenicity island termed the locus of enterocyte effacement (LEE), which encodes a type III secretion system. The LEE-encoded regulator Ler is critical for coordinate expression of gene products necessary for elaboration of the type III secretion system and the AE phenotype. The goal of this research is to elucidate the molecular mechanism of Ler-mediated transcriptional activation of EPEC virulence factors. During this period of support we will perform experiments to characterize the functional domains of Ler and investigate the "Ler-like" molecules of other Gram-negative pathogens, comparing their activities and DNA binding characteristics to those of Ler. We will correlate regulatory observations with the ability of EPEC to cause AE lesions on human epithelial cells in culture. EPEC and *E. coli* serotype O157:H7, a member of the enterohemorrhagic *E. coli* (EHEC) category of *E. coli*, are related pathogens. Like EPEC, EHEC causes AE intestinal lesions and possesses a LEE-encoded type III secretion system, including Ler. 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. Perhaps even more urgently, diarrheagenic *E. coli*, including EPEC and EHEC, are in the NIAID Biodefense Research Priority Category B Pathogens. "Ler-like" molecules have been identified in a number of Gram-negative pathogens, and thus determining the molecular mechanism of Ler-mediated transcriptional activation in EPEC may lead to the development of effective therapies preventing disease caused by a wide range of pathogens.

**Grant:** 1R15AI054401-01A1  
**Program Director:** PETERS, N KENT  
**Principal Investigator:** SANCHEZ, SUSAN PHD  
**Title:** Ceftiofur use in cattle: a public health concern?  
**Institution:** UNIVERSITY OF GEORGIA ATHENS, GA  
**Project Period:** 2004/04/01-2007/03/31

**DESCRIPTION** (provided by applicant): The overall objective, of this proposal, is to determine if there is a link between the use of antibiotics in food animals and the presence of resistant *Salmonella* in the human population. The hypothesis is that the therapeutic use of ceftiofur in food animals is selecting for resistance to cephalosporins in members of the Family Enterobacteriaceae. Therefore, our short-term goal is to determine if the use of ceftiofur in sick cattle has resulted in resistant zoonotic salmonellosis in people. Our intention is to investigate the source of ceftiofur/ceftriaxone-resistant isolates cultured in our State Veterinary Diagnostic Laboratory and determine if they are related to ceftriaxone resistant cases of salmonellosis in humans identified by the Public Health Service in Georgia and the Centers for Disease Control. Specific aim 1. Determine whether ceftriaxone-resistant *Salmonella* isolates identified by our diagnostic laboratory are genetically related to human isolates associated with outbreak or sporadic cases of salmonellosis. The working hypothesis will be tested by determining the genetic relatedness of ceftriaxone-resistant *Salmonella* from farms and human cases by PFGE and characterizing the cephalosporinase locus using molecular techniques. Specific aim 2. Determine the prevalence of ceftiofur resistance in the normal, gram negative flora of food production animals and potential transmission of ceftriaxone resistance from animal microflora to *Salmonella*. Ceftiofur usage may amplify the gene reservoir by enriching for normal flora containing extended-spectrum cephalosporinases. The working hypothesis will be tested through molecular analysis of cephalosporinase(s) within animal microflora gathered from farms with or without a history of ceftiofur usage. We will also address the potential for plasmid transmission of ceftriaxone resistance to *Salmonella* from the animals' microflora in vitro as well as with a simulated animal production environment. By surveying the animals on the farm, we will discern whether antibiotic use on the farm influences the acquisition of drug resistance by *Salmonella* interacting with the resident microflora of cattle. This proposal seeks to involve undergraduate students in molecular epidemiology investigations to increase their exposure to biomedical research.

**Grant:** 1R15AI054402-01A1  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** PYBUS, VIVIEN PHD  
**Title:** Bacteriocins inhibiting growth of vaginal lactobacilli  
**Institution:** KALAMAZOO COLLEGE KALAMAZOO, MI  
**Project Period:** 2004/03/15-2007/02/28

DESCRIPTION (provided by applicant): Bacterial vaginosis (BV) is the leading cause of vaginal tract infection in women of reproductive age in the U.S. Evidence from epidemiological studies suggests that BV is a risk factor for serious female upper genital tract infections and preterm delivery, which is the major cause of perinatal morbidity and mortality in the developed world. The estimated cost to the U.S. of BV-related complications during pregnancy is \$500 million to \$1 billion, per annum. BV is a polymicrobial syndrome characterized by an alteration in the normal vaginal microflora, such that the *Lactobacillus*-dominated populations present in healthy individuals are replaced by *Gardnerella vaginalis*, anaerobes, and genital mycoplasmas. Currently, the mechanisms that cause this shift in the vaginal microflora are not well understood. Development of BV is generally preceded by decreased concentrations of lactobacilli; however, mechanisms which may account for the disappearance of the vaginal lactobacilli are not well documented. The overall goal of this study is to understand the pathogenesis of BV by describing microbial interactions that could lead to the decline in vaginal lactobacilli and pave the way for the establishment of a BV-associated microflora. Preliminary data collected with undergraduate student researchers in my laboratory show that certain strains of vaginal enterococci can produce a class of antibiotics known as bacteriocins, which inhibit the growth of vaginal lactobacilli in vitro. One objective of the proposed study is to chemically purify and characterize bacteriocins produced by vaginal enterococci. The in vivo significance of such antibacterial substances as a mechanism contributing to the decline of vaginal lactobacilli will be assessed using a chemostat to model the in vivo conditions of the vagina. In addition, chemical characterization of such bacteriocins may reveal novel antibacterial agents, with future potential for the control of microbial growth, including the treatment of infectious diseases. The involvement of undergraduate students in all aspects of this project will enhance the current strengths of our institution in engaging and mentoring students in the conduct and presentation of scientific research.

**Grant:** 1R15AI057382-01A1  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** TEMPLE, LOUISE M PHD  
**Title:** Bordetella pathogenesis: an undergraduate research tool  
**Institution:** JAMES MADISON UNIVERSITY HARRISONBURG, VA  
**Project Period:** 2004/07/01-2007/06/30

DESCRIPTION (provided by applicant): The broad based pathogenesis study described here accomplishes all of the AREA program objectives: (1) supporting meritorious research, (2) exposing undergraduates to research, and (3) strengthening the research environments in non-research intensive schools. The experimental model, *Bordetella avium*, causes a highly contagious respiratory disease in its avian hosts, primarily turkeys, with striking similarity to whooping cough in children, caused by *B. pertussis*. Species specificity is determined largely by the attachment process of the bacteria to the tracheal cilia of the relevant host, and pathogenic effects are limited to the upper respiratory tract of the bird. Using genetic and biochemical approaches and in vivo and in vitro models, we and our collaborators have identified 19 genes important in virulence, including those encoding surface molecules (Fha, fimbriae, and LPS). In addition, we discovered two proteins essential for hemagglutination that are different from the hemagglutinin of *B. pertussis*. In the proposed study we will use classic methods to purify and characterize these factors while continuing to define and explore the constellation of virulence factors of this organism. We will also broaden our studies by developing tissue and/or organ culture models for studying toxicity, an area that is largely unexplored for *B. avium*. The systematic examination of virulence factors in *B. avium* will yield important and useful information about that organism and the disease in turkeys, and may prove helpful in understanding disease causation among all the *Bordetella* species. Over the 3 year project period, this project will expose more than 60 students in laboratory and independent research to an excellent model of research and give them an opportunity to make unique contributions to the study of an important pathogen. Having a broad based approach provides students with numerous opportunities to explore relevant scientific questions, exert creative thinking, and learn the fundamentals of biomedical research. The ongoing collaborative study has served a catalytic role over the last eight years, increasing the interest of students and faculty alike in research, and will continue to enhance the research environment for undergraduates in the sciences at Drew University.

**Grant:** 1R15AI057408-01  
**Program Director:** PETERS, N KENT  
**Principal Investigator:** DAVIS, MICHAEL A PHD  
**Title:** Isolation of New Antibiotics From Soil Microorganisms  
**Institution:** CENTRAL CONNECTICUT STATE UNIVERSITY NEW BRITAIN, CT  
**Project Period:** 2004/04/01-2007/03/31

DESCRIPTION (provided by applicant): This project has two main goals: (1) to identify and isolate novel antibacterial compounds from natural sources (soil microorganisms), and (2) to train students in integrative approaches to research, combining microbiology and organic chemistry. Antibiotic chemotherapy has been increasingly compromised by the evolution of antibiotic resistant strains, and new antibiotic compounds are needed to replace or supplement those in current use. The original sources of many common antibiotics were soil microorganisms. This project continues that hunt, seeking bacteria that secrete novel compounds from soil samples collected from a wide range of sites. Varied laboratory growth conditions will be employed to extend the range of types of organisms cultured from these samples, including anaerobiosis and nutrient limited media. Bacterial species producing inhibitory chemicals will be identified in mixed competition cultures, then isolated and grown in pure cultures. The identity of these candidate antibiotic-producers will be determined by standard microbiological typing procedures, supplemented with molecular techniques (PFGE). Conditioned media from these pure cultures will be treated to extract and purify the active antibacterial compounds by standard and HPLC-based chromatography. The composition and structure of these compounds will be determined using spectroscopic techniques, and the results compared with known antibacterial agents. Novel compounds will be candidates for later work (not described here) determining the usefulness of these agents for disease chemotherapy. Student researchers participating in this project will experience the integration of fields normally taught as separate disciplines. Their continued success as scientists, and their contributions to advancements in human health will be enhanced by a multidisciplinary approach to research.

**Grant:** 1R15AI057437-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** WEAVER, TODD M PHD  
**Title:** Characterization of Hemolysin A from *Proteus mirabilis*  
**Institution:** UNIVERSITY OF WISCONSIN LA CROSSE LA CROSSE, WI  
**Project Period:** 2004/03/15-2007/02/28

DESCRIPTION (provided by applicant): *Proteus* species are second only to *Escherichia coli* as the most common causative agent of Gram-negative based urinary tract infections and many harbor several virulence factors that provide inherent uropathogenicity. One of the virulence factors is a hemolysin system comprised of hemolysin A (HpmA) and hemolysin B (HpmB). The hemolysin (A/B) system within *Proteus mirabilis* belongs to a two-partner secretion (TPS) pathway found in various Gram-negative bacteria. TPS pathways are responsible for the secretion of large exoproteins, including the Ca<sup>2+</sup>-independent cytolysins/hemolysins from *Serratia marcescens*, *Edwardsiella tarda*, *Yersinia pestis* and *Proteus mirabilis*. HpmA is secreted and activated by HpmB allowing *Proteus* to lyse red blood cells. The overarching goal of the research aims to determine the mode of HpmA activation. A purified N-terminal fragment of HpmA (trcHpmA) was found sufficient to activate full-length inactive hemolysin A (HpmA\*) and restore in vitro hemolysis. Both a CxxC and a deacetylase motif have been identified within the N-terminal fragment. The specific aims seek to examine the function of the CxxC, deacetylase and amidation motifs in the activation of HpmA. First, a CxxC motif was located within the amino terminal domain between cysteine 144 and cysteine 147. CxxC motifs have been shown to be involved within metal binding and disulfide isomerase sites. The research objectives will characterize the CxxC motif for both metal binding capacity and disulfide isomerase activity. In addition, two GKK amidation sequences were predicted between positions 1190 - 1193 and 1281 - 1284 of the C-terminal domain. Amidation sites have been shown to be instrumental during the activation of bioactive peptides. An additional part of the research objectives aim to analyze for proteolytic processing of HpmA via an amidation type mechanism during the activation of hemolysis. Collectively, the aims may determine if the CxxC, deacetylase, and amidation motifs have functional roles during the activation of HpmA. The results may provide information regarding the hemolysin virulence factor within *Proteus mirabilis* and allow new drug design strategies for the treatment of *Proteus*-based urinary tract infections.

**Grant:** 1R01DE014685-01A2  
**Program Director:** KELTY, MIRIAM F.  
**Principal Investigator:** SCANNAPIECO, FRANK A. DMD  
**Title:** Oral Health and Ventilator Associated Pneumonia  
**Institution:** STATE UNIVERSITY OF NEW YORK AT AMHERST, NY  
BUFFALO  
**Project Period:** 2004/07/01-2007/06/30

DESCRIPTION (provided by applicant): Recent studies have found that poor oral hygiene may foster the colonization of the oropharynx by potential respiratory pathogens in mechanically-ventilated (MV), intensive care unit (ICU) patients. Thus, improvements in oral hygiene in MV-ICU patients may prevent ventilator-associated pneumonia (VAP). The Specific Aims of this revised application are: 1) to organize the necessary infrastructure to develop and perform a pilot clinical trial to evaluate alternative oral hygiene procedures to prevent VAP; 2) to use this organization to perform a pilot clinical trial to determine if the use of oral topical chlorhexidine gluconate (CHX) will prevent dental plaque, oropharyngeal colonization by respiratory pathogens, and VAP in MV-ICU patients. This pilot longitudinal, double blind intervention study will consider the appropriate frequency of delivery of CHX to improve oral hygiene in MV-ICU patients. Preliminary data from these pilot studies will also allow accurate sample size calculations to be made for a large scale multi-center clinical trial; and 3) to perform molecular epidemiological studies to genetically type bacterial strains cultured from lower airway secretions of MV-ICU patients with or without VAP and compare them to strains of the same species isolated from their dental plaque. This pilot study will enable this multidisciplinary team of investigators to organize the infrastructure, patient recruitment and methodologic protocols, and data management and analysis procedures necessary to perform a multi-center, controlled clinical trial to assess the efficacy and generalizability of this intervention to improve oral hygiene in MV-ICU and prevent VAP.

**Grant:** 1R21AI057952-01A1  
**Program Director:** FULDNER, REBECCA A.  
**Principal Investigator:** HIGH, KEVIN P MD  
**Title:** Brucella, Aging and Role of IL-17 in Host Defense  
**Institution:** WAKE FOREST UNIVERSITY HEALTH SCIENCES WISNTON-SALEM, NC  
**Project Period:** 2004/07/01-2006/06/30

DESCRIPTION (provided by applicant): Adults over the age of 65 comprise the fastest growing segment of the U.S. population. Aging increases susceptibility to most intracellular microbes (e.g. Mycobacterium, Salmonella, influenza and other viruses), likely due to waning immunity with advanced age termed 'immune senescence'. Immune senescence is characterized by impaired Th1 immunity, and efforts to reverse the responses that wane in immune senescence have been largely unsuccessful. However, recent data suggest augmenting immune responses that remain intact, even in far advanced age, may be a more achievable strategy to reduce the burden of infectious diseases in older adults. Brucella spp., important causes of disease in both human and animals, represent an exception to the rule that age increases the risk of infection due to intracellular pathogens. Scant published literature and our preliminary experiments suggest older adult mice and humans are no more susceptible to Brucella infection, and may in fact be less susceptible to this pathogen. In this proposal, we will use murine models to explore the immune mechanisms that remain intact or are enhanced with age, that allow efficient clearance of Brucella infection. Our preliminary data demonstrate marked increases in the poorly studied T cell cytokine IL-17 in response to whole Brucella organisms and specific Brucella antigens, particularly in older mice when compared to young adult mice. We suspect that the enhanced resistance of older adult mice may be due to IL-17, and the first aim of this proposal is to better define this association across the age spectrum. Very recent data have suggested IL-17 responses may be mediated by the antigen presenting cell-derived cytokine IL-23. This recently discovered feedback loop at the innate/adaptive interface may be an IL-12 independent mechanism to activate Th1 immune responses, a critical need to enhance resistance to intracellular pathogens in older adults. Initial investigations in this regard form the basis of our second aim. Finally, we will determine the clinical relevance of IL-17 in host defense by blocking the activity of this cytokine in murine models of brucellosis, and assessing the presence of IL-17 secreting memory T cells in humans with active or past Brucella infection. The data generated in this proposal will form the foundation of future R01 proposals aimed at enhancing immunity vs. intracellular pathogens in our aging population.



**Grant:** 2R01DC000263-17  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** CHOLE, RICHARD A  
**Title:** Cellular and Molecular Biology of Cholesteatoma  
**Institution:** WASHINGTON UNIVERSITY ST LOUIS, MO  
**Project Period:** 1985/09/16-2008/11/30

DESCRIPTION (provided by applicant): Cholesteatomas of the middle ear and mastoid develop as a complication of otitis media. These epidermal structures often become infected with a mixture of aerobic and anaerobic bacteria, the most common of which is *Ps. aeruginosa*. Bacteria form biofilms within cholesteatomas resulting in chronic infection. These infected cholesteatomas are aggressive and cause increased osteolysis. We have identified strains of otopathogenic *Ps. aeruginosa* (OPPA) that have increased adherence to keratinocytes and increased biofilm production. We will further characterize these isolates by examining the expression of quorum sensing genes and alginate gene expression and production. We propose to study the pathogenesis and virulence of these chronic infections. With regard to pathogenesis, we will study the adherence of these organisms to keratinocytes using randomly directed mini-Tn5 transposon mutagenesis of adherent OPPA isolates. The mutagenesis screen will be enriched for non-adherent mutant bacteria and will subsequently be sequenced to identify novel adhesin genes. With regard to virulence, preliminary studies show that our OPPA strains produce osteoclastogenesis by both an LPS-dependent and LPS-independent mechanism. LPS-dependent studies will be done with LPS sensitive murine osteoclast precursors. Expression of signals associated with osteoclastogenesis (e.g. RANKL, TNFa) will be determined to understand the mechanism of *Ps. aeruginosa* LPS mediated osteoclastogenesis. To study LPS-independent osteoclastogenesis, we will use LPS-insensitive osteoclast precursors derived from mice deficient in toll-like receptor 4 to determine which portions of the osteoclast development pathway are induced.

**Grant:** 2R01DC002148-12A2  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** EHRLICH, GARTH D  
**Title:** Molecular Analysis of Pathogens in Otitis Media by PCR  
**Institution:** ALLEGHENY-SINGER RESEARCH INSTITUTE PITTSBURGH, PA  
**Project Period:** 1993/12/01-2009/03/31

DESCRIPTION (provided by applicant): Otitis media (OM) is the most common reason that an ill child visits a health care provider or undergoes a general anesthetic. OM is also the most common reason that a child receives an oral antibiotic and the over-treatment of patients with OM has been suspected of contributing to the development of antimicrobial-resistant organisms. OM disproportionately affects socio-economically disadvantaged children, Native American children and is a factor that inhibits women from full participation in the workforce. The major focus of our laboratory for the past decade has been elucidating the path physiology of chronic OM with the ultimate goal of developing more effective treatments. During this time, we have shown that chronic OM is not a purely inflammatory process, but rather is a bacterial biofilm illness. The recognition that OM is a biofilm disease then led to a novel hypothesis: The Distributed Genome Hypothesis. This hypothesis states that there is a supra-genome for pathogenic bacteria and that each individual bacterium possesses only a subset of genes from the supra-genome. The supra-genome is a reservoir for panoply of contingency genes that collectively provides a significant survival benefit for the population-at-large. In this continuing application we specifically test the Distributed Genome Hypothesis in *Haemophilus influenza* (HI) with four Specific Aims: 1) Perform comparative genomic studies among clinical isolates of HI To characterize the extent of genomic plasticity; 2) Determine the extent of HI inter-isolate recombination during the infectious process; 3) Prepare transformation knockouts of HI and compare their survival time in vivo with wild-type congener strains; 4) Phenotypic characterization of HI biofilms. These Specific Aims will be accomplished by experiments using state-of-the-art high throughput genomic, molecular biology, imaging and modeling techniques, as well as investigations in children. Preliminary data generated from a micro array library composed of 10 clinical isolates of *H. influenza* demonstrated that *H. influenza* does indeed have a supra-genome that is twice the Size of a single bacterium. These findings shed light on many aspects of OM, including disease persistence in the face of antibiotic treatment, and provide an explanation for the success of adenoidectomy in the management of OM.

**Grant:** 2R01DC003915-06  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** BAKALETZ, LAUREN O PHD  
**Title:** Determinants of H. influenzae Virulence in Otitis Media  
**Institution:** CHILDREN'S RESEARCH INSTITUTE COLUMBUS, OH  
**Project Period:** 1999/09/30-2009/08/31

DESCRIPTION (provided by applicant): Middle ear infection or otitis media (OM) is a highly prevalent pediatric disease worldwide. There were nearly twenty-five million physician's office visits made for OM in 1990, and available evidence suggests that the incidence is increasing. While only very rarely associated with mortality, the morbidity associated with OM is significant. The socioeconomic impact of OM is also great. Direct and indirect costs of diagnosing and managing OM exceed \$5 billion annually in the U.S. alone. Clearly, there is a tremendous need to develop more effective and accepted approaches to the management and preferably, the prevention of OM. Vaccine development holds the greatest promise and would be the most cost-effective method to accomplish this goal. However, progress in terms of vaccine development for nontypeable *Haemophilus influenzae* (NTHI), the Gram-negative pathogen that both predominates in chronic otitis media with effusion or OME, as well as being a significant etiologic agent of acute OM, continues to be hampered by our incomplete understanding of the pathogenesis and immunobiology of OM, a polymicrobial disease caused by one or more of the three predominant bacterial pathogens, whose ability to invade the tympanum is facilitated by virtually any upper respiratory tract (URT) virus. During the past 4 years, we have: sequenced and will soon complete the annotation of the genome of an OM isolate of nontypeable *Haemophilus influenzae* (NTHI); developed and used DNA plasmid based microarrays to conduct strain comparison studies for two clinical OM isolates; developed a promoter trap system in NTHI with which we have monitored gene expression in vivo, during experimental OM; and have developed a signature tag mutagenesis system in NTHI that provided us with a complementary system to identify genes that are essential for colonization and induction of OM. Not only are all these tremendous resources available to us to capitalize upon as we extend our studies of the pathogenesis of NTHI-induced OM, but they have also already resulted in the identification of multiple potential new virulence determinants for NTHI. We propose experiments for the next funding period designed to continue to enhance our understanding of both NTHI pathogenesis in OM at the molecular level as well as further our investigation of a focused group of novel virulence determinants. We will first rigorously assess the feasibility of using lux-expressing NTHI strain 86-028NP to provide a non-invasive, whole animal imaging system to be applied to, and significantly advance, our studies of both pathogenesis and vaccine-mediated prevention of OM. Secondly, we will use a chinchilla super infection model to assess the protective efficacy of one well-developed candidate antigen and one novel and highly promising, but less developed candidate, for ability to prevent ascending OM after intranasal immunization. We will also continue to identify and characterize putative virulence determinants and assess their potential as vaccine or therapeutic targets via a variety of methodologies, including gene expression profiling by microarray analysis. Overall, our studies will lead to an improved understanding of OM caused by NTHI and lead to new strategies to prevent OM.

**Grant:** 1R01DE014371-01A2  
**Program Director:** ROTHERMEL, ANNETTE L.  
**Principal Investigator:** COSTALONGA, MASSIMO PHD  
**Title:** Tracking Mucosal T cells to Commensal Microbes in Vivo  
**Institution:** UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN  
**Project Period:** 2004/02/01-2007/01/31

**DESCRIPTION:** IgA antibodies provide an important first line of defense against mucosal pathogens. The induction of immunoglobulins against T cell-dependent antigens is subordinate to T cell activation, T/B cell interaction and cytokines. Our long-range goal is to learn how the mucosal immune system responds to protein antigens of the virulent and commensal microbiota. Our current objective is to determine, in vivo, which type of T cell response is induced by the commensal *Lactobacillus murinus*, while transiting the intestine. We hypothesize that intestinal commensal microorganisms transmucosally induce a T cell-dependent humoral response, while suppressing the cell-mediated response. Testing this hypothesis can lead to the design of effective microbial delivery systems. We will test this hypothesis by determining 1) how a commensal microbe primes naive T cells in the intestine and 2) the type of cytokines elicited in memory T cells. We engineered *L. murinus* to express an ovalbumin epitope that induces ovalbumin-specific T cell proliferation when injected subcutaneously. We inject a small population of ovalbumin-specific T cells into recipient mice and feed *L. murinus* in high numbers. Ovalbumin-specific T cells are tracked by staining cell suspensions or tissues of the recipients with anti-CD4 and an anti-T cell receptor monoclonal antibody. Four-color flow cytometry and confocal immunohistology will establish in vivo that commensal microorganisms transiting the intestine activate T cells to induce CD69 expression. In an antigen-specific manner we will test the phenotype and kinetics of transmucosal T cell activation, proliferation and differentiation into cytokine-producing memory cells. Collectively, this research will elucidate the missing link between the oral antigen delivery and the production of secretory antibodies. The data will be important to human health, establishing a framework to study in vivo mucosal infectious agents and oral vaccines.

**Grant:** 1R01DE014868-01A2  
**Program Director:** GIOVANNI, MARIA Y.  
**Principal Investigator:** GILL, STEVEN R PHD  
**Title:** Community Genomics of the Human Oral Microbiome  
**Institution:** INSTITUTE FOR GENOMIC RESEARCH ROCKVILLE, MD  
**Project Period:** 2004/08/16-2007/07/31

**DESCRIPTION:** The human oral cavity is home to a complex community of >700 microorganisms with a central role in oral health and disease. In our application, this oral microbial community, or oral microbiome, is viewed as a single dynamic entity, with variation in its behavior and composition across space (different oral niches in different individuals), time, and with perturbation. Despite the critical importance of this community in maintaining human health and provoking oral disease, the genetic basis for its behavior has not been well explored. The long-term objectives of this application are to develop a comprehensive understanding of the oral microbial community at the genomic level so that oral health can be promoted, and disease ameliorated or prevented. We will address these goals by characterizing relative gene and genome abundance and gene expression in the oral microbiome in subjects with healthy periodontal and mucosal tissues, and then extend our analysis to the oral microbiome associated with chronic periodontal diseases. Data from this application will not only be used to build a database for genes in the oral microbiome, but will also lead to new experimental paradigms for exploring gene abundance and expression, and examining the interaction of the microbiome with the human host. The Specific Aims of this application are: Aim 1. Characterize the gene and genome content of the healthy human oral microbiome. Our approaches include high-throughput sequencing of random and targeted plasmid and fosmid genomic libraries and classification of functional genes and gene families. Aim 2. Characterize the gene and genome content of the oral microbiome associated with chronic periodontitis. Aim 3. Development of community genomic tools for oral microbiome data. An oral microbiome gene database will be created for use by the scientific community. A high-density DNA microarray with approximately 2,000 genes identified in Aims 1 and 2 will be developed with the ultimate goal of identifying patterns of gene abundance and expression that predict clinical natural history and response to manipulation. Among the expected long-term benefits of this work will be a revolutionized understanding of the human oral ecosystem and new approaches for classification and management of oral diseases.

**Grant:** 1R01DE015124-01A2  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** KAPLAN, JEFFREY B PHD  
**Title:** Biofilm growth and detachment of an oral pathogen  
**Institution:** UNIV OF MED/DENT NJ NEWARK NEWARK, NJ  
**Project Period:** 2004/07/01-2008/03/31

**DESCRIPTION:** Biofilms are communities of bacteria growing attached to a surface. Biofilms are responsible for more than 80% of bacterial infections in humans. Examples of diseases caused by biofilms include Dental caries, periodontitis, cystic fibrosis pneumonia, and infective endocarditis, and infections of various medical devices such as intravenous catheters, artificial joints and contact lenses. Little is known about the detachment of bacteria from biofilms, a process necessary for the spread of infections to new sites. Biofilm detachment represents an important area of future research that is expected to lead to novel strategies for treating biofilm infections. The Gram-negative oral bacterium *Actinobacillus actinomycetemcomitans* has been implicated as the causative agent of localized juvenile periodontitis, a severe and rapid form of periodontal disease that affects 70,000 primarily African-Americans in the U.S. annually. *A. actinomycetemcomitans* also causes several non-oral infections including bacteremias, brain abscesses and infective endocarditis. A striking feature of fresh clinical isolates of *A. actinomycetemcomitans* is their ability form extremely tenacious biofilms on surfaces such as glass, plastic and saliva-coated hydroxyapatite, a property that has been shown to be essential for virulence in a rat model. Tight adherence to surfaces also makes *A. actinomycetemcomitans* an excellent model for studying biofilm growth and detachment in vitro. Genetic and microscopic studies in this laboratory have shown that *A. actinomycetemcomitans* cells grown attached to surfaces in broth form highly-differentiated biofilm colonies that are capable of releasing cells into the medium. Biochemical and genetic studies indicate that *A. actinomycetemcomitans* biofilm colonies are held together by a sticky, extracellular polysaccharide. The proposed experiments are a continuation of our preliminary studies which have identified an enzyme produced by *A. actinomycetemcomitans* which causes the degradation of the sticky polysaccharide coating and detachment of *A. actinomycetemcomitans* cells from the biofilm aggregate. We plan to use genetic techniques to understand how production of this enzyme is regulated in the bacterial cell, and biochemical techniques to determine the structure of the polysaccharide substance on the surface of the cell. Preliminary data indicate that this enzyme is capable of degrading biofilms produced by other species of Gram-negative and Gram-positive bacteria, indicating that it may represent a novel anti-biofilm therapeutic with broad spectrum potential.

**Grant:** 1R01DE015254-01A2  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** HAJISHENGALLIS, GEORGIOS DDS DENTISTRY  
**Title:** Pattern Recognition of *P. gingivalis* Virulence Factors  
**Institution:** LOUISIANA STATE UNIV HSC NEW ORLEANS NEW ORLEANS, LA  
**Project Period:** 2004/07/01-2008/06/30

**DESCRIPTION:** Toll-like receptors (TLRs) and other pattern-recognition receptors (PRRs) form functional receptor complexes that recognize pathogen-associated molecular patterns (PAMPs). Activation of the TLR signaling pathway by PAMPs leads to induction of immune and inflammatory responses. *Porphyromonas gingivalis* is an important pathogen in human periodontitis. A major cell surface component of this oral pathogen is the fimbriae, which function as an adhesin. Strikingly, fimbriae activate transcription factor NF-KappaB and induce production of proinflammatory cytokines through interactions with several PRRs. Understanding the molecular basis of how the host recognizes and responds to *P. gingivalis* fimbriae is essential for developing molecular approaches to control periodontal inflammation. Therefore, the objective of this grant is to elucidate the proinflammatory interactions of fimbriae with PRRs. The application proposes that fimbriae function as a PAMP and interact in a regulated mode, and through discrete epitopes, with different binding PRRs resulting in the activation of proinflammatory TLR signaling. Fimbrial epitopes involved in cellular binding and/or activation will be identified using fimbrial peptides and mutant fimbriae. Importantly, epitopes involved in binding but not activation may find application as antagonists of fimbria-induced inflammation. Experiments in our laboratory have shown that TLR2 and TLR4 mediate fimbria-induced signaling, but initial recognition of fimbriae is mediated by a cooperation between CD14 and CD11 b/CD18, which thus appear to serve as TLR co-receptors. It is posited that fimbriae initially bind to CD14, and the fimbriae/CD14 complex induces TLR2-mediated "inside-out" signaling that leads to activation of the ligand-binding capacity of CD11b/CD18. PRR-fimbriae interactions will be examined in human monocytes and mouse macrophages derived from normal and PRR-deficient mice. Elucidation of the mechanisms whereby PRRs recognize and respond to fimbriae and identification of fimbrial antagonists may facilitate the design of novel approaches to therapeutic intervention in both periodontitis and atherosclerosis, where *P. gingivalis* has also been implicated.

**Grant:** 1R01DE015720-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** KESAVALU, LAKSHMYA N DVM  
**Title:** Oral Pathogens: Polymicrobial Virulence Interactions  
**Institution:** UNIVERSITY OF KENTUCKY LEXINGTON, KY  
**Project Period:** 2004/04/01-2006/12/31

**DESCRIPTION:** The predominant polymicrobial infection of mankind is expressed clinically as periodontal disease, which afflicts nearly one-half of the population by 50 years of age, and is related to development of a microbial biofilm colonizing the subgingival sulcus. The suggested mechanisms of pathogenesis are varied, in most part due to the complex microbial community consisting of numerous bacterial taxa, viruses, and fungi. Nevertheless, certain of these subgingival microbial consortia are consistently correlated with a progressive destruction of soft and hard tissue that have been well documented to occur in clinical settings (i.e., periodontitis). Various in vivo and in vitro investigations have suggested that the dominance of selected species in the subgingival ecology results from both microbial synergistic and antagonistic relationships. These have been linked to the nature of available surfaces for colonization, available nutrients, and physiologic "food webs" that exists within the community. Molecular microbiologic studies have described nearly 500 species of bacteria that can inhabit this ecological niche, although several specific microbial complexes have been described at sites of progressing tissue destruction. A predominant consortia identified in a majority of adult periodontitis patients consists of *Porphyromonas gingivalis*, *Tannerella forsythensis* [*Bacteroides forsythus*], and *Treponema denticola*. The correlation of this consortium with disease has been proposed to result from synergistic physiological, host evasion, and/or tissue destructive capabilities among the component species. The objectives of this R01 application are to test a hypothesis that this polymicrobial consortium comprises a "virulence web" that synergistically increases tissue destructive host responses, and the consortia to be less effective modify that host immune responses. Three Specific Aims are proposed using an animal model system to test this hypothesis: (1) To determine molecular interbacterial synergistic virulence effects of *P. gingivalis*, *T. forsythensis*, and *T. denticola* in an in vivo calvarial bone resorption model, (2) To determine the characteristics of acquired humoral immune responses to a polymicrobial infection and the ability of this response to modulate in vivo calvarial bone resorption, and (3) To determine the characteristics of active humoral immune responses to polymicrobial immunization and ability of this response to modulate bone resorption. The long-range goals from this study will be to document microbial interactions, virulence synergisms, characterize both acquired and active immune responses, and relate these to alterations in tissue destruction and bone resorption. The significance of this application is that clinical observations have shown the ability of oral microorganisms to translocate into the circulation and manifest systemically as endocarditis, brain/kidney/lung, and intra-abdominal infections and contributing to risks of diabetes, coronary artery disease, osteoporosis, obesity, and preterm birth. Consequently, the host response to these chronic infections must be considered as critical to general health.



**Grant:** 1R01DE016133-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** KACHLANY, SCOTT C PHD  
**Title:** Leukotoxin production by *A. actinomycetemcomitans*  
**Institution:** UNIV OF MED/DENT NJ NEWARK NEWARK, NJ  
**Project Period:** 2004/07/01-2009/03/31

**DESCRIPTION:** *Actinobacillus actinomycetemcomitans* is a bacterium that is the etiologic agent for localized aggressive periodontitis (LAP). LAP is a destructive and aggressive disease of the oral cavity that affects adolescents. The incidence of LAP varies among population groups, but afflicts minorities and the underprivileged at a higher frequency. Failure to treat LAP results in the loss of teeth and other health-related problems. In addition, *A. actinomycetemcomitans* is part of the HACEK group of bacteria that causes infective endocarditis, a disease of heart valves and tissue. *A. actinomycetemcomitans* secretes a protein toxin known as leukotoxin. Leukotoxin destroys leukocytes of humans, and likely plays a significant role in the pathogenesis of *A. actinomycetemcomitans* by helping the bacterium evade the immune response. Leukotoxin is an RTX (repeats in toxin) toxin that includes other important toxins such as *E. coli* alpha-hemolysin, *M. haemolytica* leukotoxin, *B. pertussis* adenylate cyclase, and *V. cholerae* RTX toxin. To date, little is known about how *A. actinomycetemcomitans* leukotoxin is produced, activated, and secreted from bacterial cells. In addition, none of the RTX toxins have been crystallized to have their three-dimensional structures solved. Proposed here are experiments that will (1) identify the genes that are required for production of active leukotoxin, (2) study the genes and proteins using genetic and biochemical approaches, and (3) grow crystals of leukotoxin and solve its three-dimensional structure at the atomic level. We expect this work to lead to a better understanding of leukotoxin production and how the toxin contributes to disease. The structural information gained through these experiments will shed more light on the mechanism of action of this important class of toxins. This new information may lead to the design of therapeutic agents that can disrupt leukotoxin activity and ultimately treat or prevent disease.

**Grant:** 2R01AR042541-10A2  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** HUDSON, ALAN P  
**Title:** Reiter's syndrome mechanism of chlamydial pathogenesis  
**Institution:** WAYNE STATE UNIVERSITY DETROIT, MI  
**Project Period:** 1993/09/30-2009/03/31

DESCRIPTION (provided by applicant): Genital infection with the bacterial pathogen *Chlamydia trachomatis* is associated with development of reactive arthritis (ReA). While it is clear that the process leading to joint disease is partly immunopathogenic in nature, the means by which *C. trachomatis* initiates and maintains that process remain to be elucidated. Data from this group and others have shown that synovial *Chlamydiae* display unusual metabolic and transcriptional characteristics and are arrested at a late stage of the developmental cycle. *Chlamydiae* displaying these and other unusual biologic attributes *in vivo* are designated to be in the persistent state. Accumulating data further indicate that persistent *C. trachomatis* cells interact in an overt but poorly understood manner with their host cells. The key to development of effective therapies to treat *Chlamydia*-associated ReA lies in understanding the biology of chlamydial persistence, and the means by which host and pathogen interact during establishment of that state. In the studies proposed here, we define the genes and gene sets from *C. trachomatis* that are involved directly or indirectly in establishment and maintenance of the persistent state, using a well-characterized *in vitro* model of chlamydial persistence. We also, and coordinately, define the changes in expression for specific, targeted sets of host genes as a function of establishment of persistent chlamydial infection in the *in vitro* model of persistence. The gene sets to be targeted in these analyses will include those from the immune system, the signal transduction system, the energy transduction system, and others. Together, these studies will provide critical new insight not only into chlamydial proteins required for persistence, but also into previously unaddressed interactions between *C. trachomatis* and its primary host cells. Using information gained from these studies, we will determine the molecular genetic basis for differences between patients who progress to chronic disease and those who do not following genital chlamydial infection, and we define the molecular basis for the remittingrelapsing phenotype of patients with chronic *Chlamydia*-induced arthritis, and. These latter studies will give important information relating to host-pathogen interaction during various stages of disease progression. Taken together, the results of the studies proposed here will provide a comprehensive understanding of chlamydial persistence and host-pathogen interaction in ReA and therefore will form the foundation for design and implementation of rational strategies to treat the disease.

**Grant:** 2R01AR043521-11  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** WEIS, JANIS J  
**Title:** Molecular genetics of Lyme arthritis susceptibility  
**Institution:** UNIVERSITY OF UTAH SALT LAKE CITY, UT  
**Project Period:** 1994/09/30-2009/05/31

DESCRIPTION (provided by applicant): Lyme disease is caused by infection with the tick-transmitted spirochete *Borrelia burgdorferi*. Infection in humans can cause inflammatory arthritis in approximately 60% of those not treated at the time of the tick bite, and this can progress to chronic disease in a small percentage of susceptible individuals. The infection-associated arthritis can be studied in mice; with strong evidence suggesting that the severity of *B. burgdorferi* induced arthritis is regulated by the genetics of the host. Using intercross populations of mice developed by crossing inbred strains that develop severe Lyme arthritis (C3H) with those that display milder arthritis (C57BL/6), we have identified six Quantitative Trait Loci (QTL) that regulate arthritis severity. These QTL are highly significant, with LOD scores ranging from 3.5-10.2. Congenic lines have been developed to isolate individual chromosomal intervals associated with QTL by backcrossing seven generations to the reciprocal parent. Several interval specific congenic lines with highly penetrant Lyme arthritis phenotypes have been generated. The goals of this application are to 1) complete the characterization of the C3H x C57BL/6 congenic lines and develop interval specific recombinant lines to narrow the physical region associated with each QTL to that amenable to positional cloning; 2) determine if *Ncf1*, a component of the phagocyte NADPH oxidase, is a candidate for a highly significant QTL mapping to chromosome 5 (Bb2); and 3) determine the mechanism by which reactive oxygen intermediates suppress *B. burgdorferi*-induced arthritis in mice. These studies will provide information on the genetic regulation of Lyme arthritis, with the ultimate goal being the identification of polymorphic genes responsible for differences in severity in mice and humans. Studies with a strong candidate gene, *Ncf1*, have further revealed an unexpected paradigm in inflammatory regulation, suggesting that reactive oxygen intermediates may play a regulatory role in Lyme arthritis.

**Grant:** 1R01AR049812-01A2  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** WASHBURN, LEIGH R  
**Title:** Membrane Proteins and Mycoplasma arthritis  
**Institution:** UNIVERSITY OF SOUTH DAKOTA VERMILLION, SD  
**Project Period:** 2004/08/03-2007/04/30

DESCRIPTION (provided by applicant): Mycoplasma arthritis is a natural pathogen of rats. Although natural infections are now rare, experimental arthritis can be induced by intravenous or intraperitoneal injections. The resulting disease is characterized by rapid onset of acute, inflammatory polyarthritis, followed by a chronic, nonmigratory phase lasting six weeks or longer in some animals. This disease has been used extensively in the past as a model for studying human arthritis, and it remains a convenient and inexpensive system for examining certain inflammatory and immunologic aspects. This study will focus on two M. arthritis surface lipoproteins, MAA1 and MAA2, both of which contribute to adherence to host tissue, are highly immunogenic for infected animals, and induce protective immunity in rats. The central hypothesis is that these proteins are important in pathogenesis of M. arthritis-induced arthritis, possibly by mediating or facilitating adherence to rat tissues. This study will further define their roles in this process. The first aim of this proposal is to prepare genetic constructs to be used in testing this hypothesis. Mutants will be constructed in which the genes encoding both proteins are disrupted by transposon mutagenesis. These mutants will then be subjected to genetic complementation to reintroduce wild-type alleles of the disrupted genes. The second aim is to test these mutants and their genetically complemented counterparts for the ability to attach to rat cells in vitro and to induce arthritis in rats. This study will help to establish the function of these important proteins in the pathogenesis of M. arthritis-induced arthritis. This, in turn, will provide insight into how similar events may occur in inflammatory joint diseases of humans and other animals.

**Grant:** 2R01CA067529-10A1

**Program Director:** HALL, ROBERT H.

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

**Title:** Helicobacter induced hepatitis and tumorigenesis

**Institution:** MASSACHUSETTS INSTITUTE OF CAMBRIDGE, MA  
TECHNOLOGY

**Project Period:** 1995/05/01-2009/06/30

DESCRIPTION (provided by applicant): Since our isolation and naming of *H. hepaticus* and elucidating it causes hepatitis and hepatocellular carcinoma in A/J, AGAFx, B6C3F1 and AXB mice, several additional novel helicobacters have been identified in hepatobiliary tissue of both humans and animals. To continue dissecting the pathogenesis of these novel enterohepatic helicobacters, we have recently completed sequencing the genome of the prototype of these emerging enterohepatic pathogens, *H. hepaticus*. Using this invaluable sequence data, we can study in detail, with the use of isogenic mutants how different virulence genes play a role in chronic inflammation and tumor induction. Furthermore, given *H. hepaticus* recognized tumor promoting ability with hepatocarcinogens and the increasing recognition of helicobacters in diseased liver tissue of humans where hepatitis B and C virus infection is endemic, we want to ascertain whether *H. hepaticus* can synergize with viral proteins to induced hepatocellular carcinomas. Finally, given the ecological niche of these helicobacters in liver is the bile canaliculi, we will explore how helicobacter products or putative virulence genes of *H. hepaticus* affect colonization dynamics and physiological properties of bile in vivo. *Helicobacter hepaticus* induced hepatitis and hepatocellular carcinoma in laboratory mice provides a powerful model system for the study of human liver disease and liver cancer. Like the human conditions, disease in the mouse model is multifactorial, with genetic and environmental factors contributing to pathogenesis. The complexity of the system necessitates parallel investigation into bacterial virulence factors, gene expression changes in liver tissue, adaptive immune changes driven by cytokines, biochemical changes in bile and acute phase response components in plasma, and of course lesion development in the liver. This proposal takes advantage of cutting-edge technology to investigate each aspect of disease pathogenesis in this model. A long-term goal is to integrate information gained in each of these areas to build a complete picture of the molecular and cellular mechanisms involved in hepatitis and hepatocellular carcinoma. In this way, our focus will shift over the next 5 years from individual Specific Aims to synergistic areas of overlap between candidate bacterial virulence factors, bacterial expression profile analysis, host expression profile analysis (at the cell, tissue, and organ level), and proteome analysis (using immunohistochemistry, western analysis, and analytic biochemistry). A more complete understanding of the etiopathogenesis of hepatobiliary disease and hepatocellular carcinoma is likely to result in new strategies for treatment and prevention of these important human diseases.

**Grant:** 1R15AI057511-01A1  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** RUNYEN-JANECKY, LAURA J PHD  
**Title:** Virulence role and regulation of Shigella suf genes  
**Institution:** UNIVERSITY OF RICHMOND RICHMOND, VA  
**Project Period:** 2004/09/01-2007/08/31

DESCRIPTION (provided by applicant): Shigella species are the causative agents of shigellosis, which results in approximately 600,000 deaths per year. Transmission usually occurs via contaminated food or water, or through person-to-person contact. Basic research aimed at characterizing the genes that Shigella uses to adapt to the human host will be useful in designing effective therapies and vaccines. Furthermore, since Shigella virulence depends largely on its ability to live inside human colon cells, a complete understanding of the mechanisms that Shigella uses to survive and multiply in the intracellular environment will provide better insight into the lifestyles of intracellular pathogens. The long-term research goal is to elucidate the metabolic and physiological processes that Shigella employs to survive and multiply the eukaryotic cell. An approach to accomplishing this goal was to characterize Shigella genes that are induced when Shigella is in the eukaryotic cytoplasm. One particular locus (suf) that is induced in response to the eukaryotic intracellular environment is homologous to a locus that enhances virulence and oxidative stress survival in the plant pathogen *Erwinia chrysanthemi*. Three of the six suf genes are predicted to mediate iron-sulfur cluster synthesis. This led to the hypothesis that the Shigella suf genes mediate adaptation to the eukaryotic cytoplasm, possibly through enhanced iron-sulfur cluster metabolism and/or oxidative stress survival. The goal of this project is to characterize the *S. flexneri* suf locus, specifically the regulation of the suf genes and the contribution of the Suf system to Shigella adaptation to the eukaryotic cytoplasm. Specific aim 1 is to identify the elements that regulate suf expression in vitro and in vivo. Because the sufA gene is induced when Shigella is in the eukaryotic cytoplasm, specific aim 2 is to assess whether the Shigella Suf proteins are required for survival and/or multiplication in the eukaryotic intracellular environment. Characterization of the Shigella suf genes will provide information about the importance of the suf genes when Shigella is in the intracellular environment and will provide a better understanding of how Shigella senses and adapts to the eukaryotic intracellular environment.

**Grant:** 1R15AI060752-01  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** ROBINSON, JAYNE B PHD  
**Title:** lasB is Essential for Surface Motility in *P. aeruginosa*  
**Institution:** UNIVERSITY OF DAYTON DAYTON, OH  
**Project Period:** 2004/06/01-2007/05/31

DESCRIPTION (provided by applicant): *Pseudomonas aeruginosa* is a ubiquitous and important opportunistic pathogen that possesses an impressive arsenal of virulence factors. Elastase, a metalloprotease encoded by the *lasB* gene, contributes to virulence by degrading or inactivating tissue and immune system components. The expression of *lasB* requires both the *las* quorum-sensing (QS) system and PQS. Little is known about the role elastase plays in the life of this bacterium. Evidence that *lasB* mutant cells are severely deficient in both twitching and swarming motility was recently discovered in our lab and is presented here for the first time. Surface associated motility is essential for survival and dispersal of bacterial populations and plays a defining role in biofilm formation. The proposed research will investigate the mechanism(s) by which elastase affects these two forms of motility. Twitching motility is dependent upon PilT mediated retraction of the Type IV pilus. Pilus retraction is inhibited in *lasB* mutant cells, thus our studies will focus on the mechanism by which *LasB* affects pilus retraction. Swarming motility requires both flagella and rhamnolipids. The number and position of flagella are altered on *lasB* mutant cells. Additionally, lesser amounts of C4-homoserine lactone (HSL) are produced by *lasB* mutant cells. The production of rhamnolipids, pyocyanin, and pyoverdine are all controlled by the *rhl* quorum-sensing system for which C4-HSL is the cognate signal. The *lasB* gene itself is regulated by QS and the proposed research seeks to determine how elastase affects AHL production in *P. aeruginosa*. The pleiotropic effects of the *lasB* mutation are best addressed by a global approach involving microarray and proteomic analyses which are proposed. The results of the proposed research will lead to a greater understanding of how *LasB*, an important virulence factor that is crucial in host tissue exploitation, also contributes to surface-associated motility, biofilm formation and QS regulation in this bacterium.

**Grant:** 1R15AI060759-01  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** SPECTOR, MICHAEL P  
**Title:** Salmonella's RpoE-Regulated Starvation-Stress Response  
**Institution:** UNIVERSITY OF SOUTH ALABAMA MOBILE, AL  
**Project Period:** 2004/07/01-2007/06/30

DESCRIPTION (provided by applicant): Whether within the host or in the external environment, enteric bacteria often survive under conditions that do not support exponential growth. One factor frequently preventing growth is starvation for a carbon and energy source (C-starvation). Upon encountering such conditions, *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) undergoes a series of changes in global gene expression and physiology that we refer to is the Starvation-Stress Response (SSR). The SSR results in a cell that is capable of surviving continued, long-term starvation and exposure to a variety of other stresses. Previously, we discovered that the SSR is partially regulated by the alternate sigma factor, sigma(E), encoded by the *rpoE* gene. We showed that sigma(E) is activated and accumulates during C-starvation and that *rpoE* is essential for long-term C-starvation survival and for C-starvation-induced cross-resistances. Sigma(E)-type sigma factors control the expression of extra-cytoplasmic functions. However, the genes regulated by such sigma factors varies greatly depending upon the bacterial species. Our preliminary data indicates that the set of genes regulated by sigma(E) during C-starvation in *S. Typhimurium* is very different from the *rpoE*-regulon of *E. coli* reported in the literature. Therefore, our first goal is to identify *S. Typhimurium* genes induced in response to C-starvation in a sigma(E)-dependent manner. A few of the more interesting genes (e.g., genes unique to *Salmonella*) will be chosen for further characterization. Using knock-out mutants, we will determine the role of each gene in long-term C-starvation survival and in C-starvation-induced cross-resistance to high temperature, low pH, oxidizing agents, and antimicrobial compounds. In addition, we will further investigate the regulation of these genes by other known stress response regulators such as cAMP-CRP, ppGpp, sigma(S), Lrp, OxyR, SoxRS, Fnr, ArcAB, PhoPQ, and PmrAB. Through our collaboration with Dr. Mark Roberts (Univ. of Glasgow), we will determine the role of these sigma(E)-SSR genes in mouse virulence.



**Grant:** 2R21AI041113-05A1  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** DYBVIG, KEVIN F PHD  
**Title:** Antigenic Variation in Mycoplasmas  
**Institution:** UNIVERSITY OF ALABAMA AT BIRMINGHAM BIRMINGHAM, AL  
**Project Period:** 1997/04/01-2006/03/31

DESCRIPTION (provided by applicant): Mycoplasmas are widely distributed in nature and commonly produce diseases of considerable economic impact, yet little information is available concerning mechanisms of pathogenesis and effective methods of control are unavailable. Many mycoplasmas undergo rapid variations in surface proteins that are thought to be important to disease pathogenesis. The issue of whether variations in these mycoplasmal proteins is primarily a mechanism for immune avoidance or instead a mechanism for creating cells with varied functions (e.g., tissue tropism) has not been addressed. In the murine pathogen *Mycoplasma pulmonis*, high-frequency phenotypic variations involving changes in the highly repetitive V-1 surface antigens affect colony morphology, the susceptibility of the organism to mycoplasma viruses, the adsorption of mycoplasmas to red blood cells, and virulence. Our laboratory has shown that the V-1 antigens are encoded by a family of genes designated *vsa* (variable surface antigen). Site-specific DNA inversions serve to vary *vsa* gene expression by recombining different genes with the *vsa* expression site, resulting in the phase-variable production of the Vsa proteins. Recently, we have shown that the Vsa proteins have a role in protecting the mycoplasma from killing by complement. Our long-range goals are to understand the molecular basis and pathogenic significance of phenotypic variations in mycoplasma. The specific aims of the current proposal are to study the pathogenic significance of Vsa variation. (i) The role of Vsa variation in immune avoidance will be examined by comparing the mycoplasma populations that arise in immuno-competent and immunocompromised mice (rag and inducible nitric oxide synthase-deficient mutants). (ii) The role of Vsa variation in disease chronicity will be examined by determining whether a mycoplasmal mutant that is not capable of Vsa variation has a diminished ability to sustain a long-term infection. (iii) The role of the Vsa proteins in mycoplasma-complement interactions will be examined in a series of in vitro assays. (iv) The pathogenic significance of the susceptibility of mycoplasma cells to complement will be evaluated by determining whether sensitivity to complement killing correlates with a diminished ability to colonize the mouse respiratory tract.

**Grant:** 2R21AI044033-06  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** MAURELLI, ANTHONY T  
**Title:** Molecular Genetic Analysis of Chlamydia Pathogenicity  
**Institution:** HENRY M. JACKSON FDN FOR THE ADV ROCKVILLE, MD  
MIL/MED  
**Project Period:** 1998/12/01-2006/03/31

DESCRIPTION (provided by applicant): Bacteria of the genus Chlamydia are significant pathogens of animals and man. The diseases caused by Chlamydia spp. in man include pneumonia, endocarditis, polyarthritis, blindness, and a wide range of sexually transmitted diseases including cervicitis, salpingitis, pelvic inflammatory disease, and infertility in females; and non-gonococcal urethritis and acute epididymitis in males. Chlamydia has also been implicated as a cofactor in a variety of chronic diseases such as coronary heart disease. Despite many years of effort, the Chlamydia remain intractable to genetic analysis due to their obligate intracellular lifestyle and complex developmental cycle. No one has been able to introduce foreign DNA into this organism and achieve stable inheritance of and expression of the foreign genes. Our long-term goal is to apply the power of genetics to study the pathogenic mechanisms of Chlamydia. The aims of this proposal include the development of genetic tools for the analysis of Chlamydia pathogenesis and hypothesis-driven aims to address specific questions of Chlamydia biology. The specific aims are to: 1) develop a method for introduction, expression, and stable maintenance of foreign DNA in Chlamydia and a system for gene replacement in Chlamydia; 2) characterize the pathway for peptidoglycan synthesis in Chlamydia; and, 3) identify the transport system for uptake of essential constituents for Chlamydia growth, specifically the source of methyl donors for methyltransferases. We will employ genetic, biochemical and cell biology strategies to each aim. Success in achieving the first aim will have a significant impact on Chlamydia research by making new tools for genetic analysis of Chlamydia available. Rapid advances in our understanding of Chlamydia pathogenesis and biology as well as the ability to construct Chlamydia mutants for vaccine development will be made possible by these new techniques. Success in aim 2 will finally resolve the Chlamydia anomaly while aim 3 will attempt to resolve another apparent paradox of Chlamydia biology. Thus, these two aims will resolve some long-standing questions of Chlamydia biology and extend our knowledge of the intracellular lifestyle of this important pathogen.

**Grant:** 2R21AI044231-06  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** WEISER, JEFFREY N.  
**Title:** Bacterial phosphorylcholine and pathogenesis  
**Institution:** UNIVERSITY OF PENNSYLVANIA PHILIDELPHIA, PA  
**Project Period:** 1999/08/01-2005/07/31

DESCRIPTION (provided by applicant): We have shown that choline phosphate or phosphorylcholine (ChoP) is found on the surface of numerous bacterial species. In particular, many of the major pathogens that colonize the mucosal surface of the respiratory tract, including members of the genera *Streptococcus*, *Haemophilus*, *Neisseria* and *Actinobacillus*, express this otherwise unusual prokaryotic structure. We have relied on comparisons of isogenic ChoP<sup>+</sup> and ChoP<sup>-</sup> mutants of *H. influenzae* to define the contribution of bacterial ChoP to colonization and the pathogenesis of disease. Where possible we have extended our findings to the leading pathogen, *S. pneumoniae*. Bacterial expression of ChoP allows for mimicry of host phospholipids. For each of the above genera, choline is obtained exclusively from host sources. Since choline is also a nutritional requirement for host cells, choline depletion by bacteria may be a previously unrecognized source of cytopathic effect. Specific Aim One will examine the effect of competition for choline on host cells. Progress during the previous funding period showed that bacterial ChoP reduces susceptibility to antimicrobial peptides that target differences between host and microbial membranes. Moreover, bacterial ChoP mimicry of platelet-activating factor (PAF) allows for attachment to epithelial cells through binding to its receptor, rPAF. Since PAF is a mediator of inflammation and cell-signaling events, interaction with rPAF could render host clearance mechanisms more permissive for bacterial survival. Such an effect could explain why multiple species utilize this common receptor. Specific Aim Two will determine the effect of bacterial adherence via ChoP on this receptor and rPAF-mediated signaling events. The expression of ChoP is highly variable suggesting that in some circumstances its expression is disadvantageous for bacterial survival. We have shown that ChoP is the target of both innate (C-reactive protein, which is present on the airway surface) and adaptive (human ChoP-specific IgG2) immune responses. The effectiveness of antibody to ChoP suggests that ChoP could serve as a common target antigen for protection against respiratory tract pathogens. Specific Aim Three will determine the effects of the immune response to ChoP on colonization, since there is a selection for ChoP-expressing bacteria during carriage, including natural human carriage.

**Grant:** 2R21AI044239-06  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** SEIFERT, H STEVEN  
**Title:** Molecular Genetics of the gonococcus  
**Institution:** NORTHWESTERN UNIVERSITY CHICAGO, IL  
**Project Period:** 1998/12/01-2005/03/31

DESCRIPTION (provided by applicant): The Gram-negative bacterium *Neisseria gonorrhoeae* (the gonococcus, Gc) is the only causative agent of the sexually-transmitted disease gonorrhea. This proposal was funded to examine the mechanisms used by the Gc RecA protein to mediate recombination and repair in this human specific pathogen and to determine how gonococcal RecA differs from the *E. coli* paradigm. In the past four years, we have shown that an under appreciated *E. coli* protein, RecX, binds *E. coli* RecA and inhibits its strand exchange and co-protease activities during the SOS response by creating an inactive form of the RecA-DNA filament. We have also shown that a Gc recX mutant is reduced for the RecA-dependent processes of pilin antigenic variation, DNA transformation, and DNA repair suggesting a positive role in regulating RecA activity. During this next granting period, we will determine the mechanism by which the Gc RecX stimulates RecA-dependent processes by analyzing the effect of Gc RecX on Gc RecA activity in vitro. In the course of our investigations, we have made the novel discovery that Gc cells contain multiple chromosomes. We will quantitate how many chromosomes are carried by Gc cells and how they segregate during cell division. As an entry point into understanding the replication mechanisms that allow multiple chromosomes, we will define the Gc origin of replication. Finally, as a human-specific pathogen, Gc are not exposed to UV light and do not have an SOS system. We presume that the most common DNA damage encountered by gonococci is the oxidative burst of polymorphnuclear cells (PMNs). We have used a Gc microarray to begin to catalog the gene expression response to oxidative damage. We have observed >40 genes that are up-regulated after exposure to H<sub>2</sub>O<sub>2</sub>. We will perform additional microarray experiments to determine which genes are preferentially expressed after H<sub>2</sub>O<sub>2</sub> exposure or nonspecific DNA damage. We will mutate damage-responsive genes to determine which upregulated genes are important for survival in the face of an oxidative burst both in vitro and in the presence of PMNs. These studies will enhance our understanding of these basic molecular processes of Gc, provide insight into how this human-specific pathogen differs in its recombination and repair capabilities from *E. coli*, and provide potential targets for novel antimicrobials.

**Grant:** 2R21AI048490-05  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** SPLITTER, GARY A DVM  
**Title:** Brucella Vaccine for Bioterrorism  
**Institution:** UNIVERSITY OF WISCONSIN MADISON MADISON, WI  
**Project Period:** 2000/07/15-2005/08/31

DESCRIPTION (provided by applicant): *Brucella* spp., a gram-negative facultative intracellular bacterium, induces chronic infectious disease and is a potential pathogen for bioterrorism. *B. abortus*, *B. melitensis*, and *B. suis* are pathogenic to humans. In contrast, human infections of *B. ovis* and *B. neotomae* have not been reported, while *B. canis* human infections are rare. Although limited genome diversity exists among *Brucella* spp., these species exhibit host preference and considerable differences in virulence. However, little is known regarding the *Brucella* genes that contribute to intracellular survival and virulence. Therefore, understanding differences among *Brucella* species genomes would provide valuable clues to different pathogenic mechanisms of this species. Pathogens can be distinguished from their avirulent counterparts by specific genes or gene clusters in the genome helping to define bacterial pathogenicity. Recently, we developed a microarray of the complete *B. melitensis* genome sequence, the species highly pathogenic to humans. Hybridization of DNA from other *Brucella* species to this microarray revealed clusters (islands) in the 16M genome that were missing in the non-virulent species. Included in these islands are ORFs encoding hypothetical proteins, transporters, transposases, and transcriptional regulators that most likely aid successful establishment of *Brucella* in human infections. The genomic context of these islands suggests horizontal acquisition and variability in virulence and host preference among *Brucella* species. Now, we will create strategies to determine the importance of these newly identified *Brucella* genomic islands to bacterial pathogenicity. We hypothesize that genetic information contained within certain of these islands contributes to establishing an intracellular niche and persistence of *Brucella* within animals. Our long-term goal is to characterize at the molecular level the role of *Brucella* genes within these genomic islands for their contribution to *Brucella* pathogenicity and survival. Defining the contributions of genomic islands to *Brucella* pathogenesis will aid in developing future *Brucella* vaccines. The following specific aims are proposed: Aim 1. We will determine the contribution of genomic islands to *Brucella* pathogenicity. (a) We will delete genomic islands from virulent *B. melitensis*. (b) We will determine if genomic islands have functional importance for *Brucella* survival in macrophages. Aim 2. We will determine persistence and dissemination of mutants in mice. We will compare the in vivo dissemination and persistence of genomic island mutants to virulent *B. melitensis* using biophotonic imaging in mice. Aim 3. We will determine gene transcription of *B. melitensis* and GI mutants in macrophages as well as gene transcription by mammalian cells responding to infection. (a) We will determine transcriptional differences between virulent *B. melitensis* and GI mutants in macrophages. (b) We will identify differences in macrophage transcription following infection of mutant and virulent *Brucella*.

**Grant:** 2R21AI048499-06  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** ROOP, ROY M  
**Title:** Brucella Stationary Phase Gene Expression and Virulence  
**Institution:** EAST CAROLINA UNIVERSITY GREENVILLE, NC  
**Project Period:** 2000/07/15-2005/09/29

DESCRIPTION (provided by applicant): The host factor (HF-I) proteins of *Escherichia coli* and *Salmonella* RNA binding proteins that are required for optimal translation of the mRNA encoding the stationary phase specific alternative  $\sigma$  factor RpoS. The hfq gene encodes HF-I, and *E. coli* and *S. typhimurium* hfq mutants display essentially the same generalized stationary phase defective phenotype as *E. coli* and *S. typhimurium* rpoS mutants. Previous studies in our laboratory have shown that the *B. abortus* hfq mutant Hfq3 displays a generalized stationary phase defect in vitro, but more importantly, mutational studies have clearly established that HF-I is required for the wild-type virulence of *B. abortus* 2308 in the mouse model. In studies funded by our current award from NIAID, we have identified 18 genetic loci in *B. abortus* 2308 that are regulated by HF-I, 6 of which are required for wild-type virulence in mice. Three (ahpCD, sodC and cydAB) encode stationary phase antioxidants that protect the intracellular brucellae from oxidative damage in the phagosomal compartment. Interestingly, the virB operon (which encodes the Type IV secretion system) and the bvrRS two-component regulatory system (which regulates genes involved in maintaining cell envelope integrity) also require HF-I for normal expression in *B. abortus* 2308. The *Brucella* spp. lack a typical RpoS homolog, so the nature of the regulatory link between HF-I and stationary phase gene expression is not yet clear. Consequently, the objectives of the project described in this competing renewal application are a) to investigate the individual contributions of the *B. abortus* HF-I regulated genes dps, cfa, znuA, oppD, and bolA to stationary phase physiology in vitro and virulence in mice; b) to better define the role of HF-I in the regulation of the virB and bvrRS operons, and c) to test the hypothesis that the alternative  $\sigma$  factor RpoE2 links HF-I and stationary phase gene expression in *B. abortus* 2308, and thus serves as a "functional RpoS homolog in this bacterium. Defining the basis for HF-I mediated stationary phase gene expression in *Brucella* and elucidating the contributions of individual stationary phase gene products to successful survival and replication in host macrophages will provide us with important basic information that will be useful for the design of novel vaccine candidates and improved chemotherapeutic approaches.

**Grant:** 1R21AI052040-01A2  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** IZZO, ANGELO A PHD  
**Title:** Metalloproteinase Functon in Tuberculosis Granulomas  
**Institution:** COLORADO STATE UNIVERSITY-FORT FORT COLLINS, CO  
COLLINS  
**Project Period:** 2004/06/01-2006/05/31

DESCRIPTION (provided by applicant): Mycobacterium tuberculosis (Mtb) infects approximately one third of the world's population. In the US, there are an estimated 10 to 15 million people infected with Mtb who have the potential to develop active disease. Among otherwise healthy persons, infection with Mtb is likely to be asymptomatic. In recent years it has become clear that if these individuals become immunosuppressed, as in cases of HIV infection, Mtb infection is reactivated. The purpose of this proposal is to use a murine model of pulmonary Mtb infection to dissect the host's immune response to identify factors that promote the formation and maintenance of granulomas during chronic Mtb infection and possible mechanisms that may also cause reactivation. Expression of anti-mycobacterial immunity depends on type 1 immune cytokines such as interferon-gamma that enable the host to mount a granulomatous inflammatory response to the infection. The lung provides an excellent environment for the organism to persist, despite the presence of a continuous immune response. Matrix metalloproteinases (MMPs) are endopeptidases that degrade the extracellular matrix and have been associated with various pathogenic states. Tissue inhibitors of MMPs regulate the activity of MMPs and provide a mechanism for controlling their activity. We propose that during pulmonary Mtb infection, MMPs are a dual-edged sword, being induced by Mtb, but required by the host to form the granuloma and then down regulated to maintain the structure. Specifically, increased MMP activity causes extracellular matrix degradation/tissue remodeling, which enables Mtb to disseminate and facilitates leukocyte trafficking into infected lungs, providing the foundation for granuloma formation. Finally, MMP down regulation is critical for fibrosis formation that is essential for granuloma stability. The MMPs that function during this process are macrophage-derived MMP-2, MMP-9 and MMP-12, which play significant roles in the disease process, being regulated directly by the virulent organism. Understanding how the immune response modulates MMP activity and produces tight, well-formed granulomas will provide insight into mechanisms that could accelerate and stabilize the natural healing process. It is intended that the information obtained from these investigations will provide a better understanding of the immunopathogenesis of tuberculosis and therefore allow for the development of better treatment regimens particularly during reactivation of infection that can be used in association with conventional anti-tuberculosis therapies.

**Grant:** 1R21AI053235-01A1  
**Program Director:** PETERS, N KENT  
**Principal Investigator:** MCNAMARA, PETER J PHD  
**Title:** S. aureus SarU and Rot regulate virulence factor genes  
**Institution:** UNIVERSITY OF WISCONSIN MADISON MADISON, WI  
**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (provided by applicant): Historically, antimicrobial therapy dramatically reduced the mortality of Staphylococcus aureus infections. Because of multidrug resistance, successful treatment of S. aureus can be difficult to achieve. Novel therapeutic interventions are desperately needed. One promising approach is to develop drugs that target the regulators of virulence factor (VF) expression. A detailed molecular mechanism of action will greatly benefit the search for an appropriate target for regulator-specific drugs. VF regulation in S. aureus is controlled by the cooperative and redundant action of the products of many loci including a family of at least six MarR-family transcriptional regulators, the SarA-homologues. This proposal focuses elucidating the molecular mechanism of two SarA-homologues, Rot and SarU. Genetic evidence suggests that Rot and SarU have a reciprocal effect on the expression of VFs. Using alpha-toxin as an example we will define the molecular interactions that lead to Rot acting as a repressor of the gene encoding alpha-toxin (hlalpha) and SarU acting as an activator of hla transcription. Our hypothesis is that Rot is a constitutively expressed repressor that directly downregulates transcription of hlalpha. This repression is relieved by RNAIII, a riboregulator that is upregulated by both a direct interaction between SarU and the agr promoters and an indirect interaction of SarU on the sarA promoters. To test this hypothesis, we propose: Aim 1. To characterize mechanism(s) of Rot repression of a-toxin production that is antagonized by RNAIII. Aim 2. To characterize mechanism(s) of SarU activation of a-toxin production by demonstrating direct and temporally appropriate interactions between SarU and its target genes.



**Grant:** 1R21AI053343-01A1  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** SCHIFFERLI, DIETER M DVM  
**Title:** Function and immunogenicity of *Yersinia pestis* fimbriae  
**Institution:** UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA  
**Project Period:** 2004/01/01-2005/12/31

DESCRIPTION (provided by applicant): *Yersinia pestis* is the causative agent of Plague. Aerosolized *Y. pestis* is feared as a most dangerous bioweapon. There is currently no vaccine protecting humans against pneumonic Plague, the most contagious and lethal form of Plague. The long-term goal of this project is to develop a new, cost-effective vaccine capable of inducing mucosal and systemic immunity for extended protection against Plague. The studied major prototype vaccines against *Y. pestis* are based on only two subunit antigens, one not required for virulence. Such vaccines might not protect a sufficiently broad population of individuals with different genetic backgrounds. They also run the risk of becoming ineffective against naturally selected or engineered mutants of *Y. pestis*. The recently deciphered *Y. pestis* genome will be used to study additional immunogenic protein targets, preferably involved in some aspects of *Y. pestis* pathogenesis. The fimbrial proteins found in most if not all Enterobacteriaceae, including *Y. pestis*, are bestowed with such properties. Fimbriae are polymeric proteins assembled as hair-like organelles on bacterial surfaces. In addition to being among the best studied and most effective bacterial immunogens, many fimbriae are directly involved in mediating bacterial binding to host mucosal surfaces. Fimbriae induce typically high titers of both anti-bacterial and antiadhesive antibodies. They can be isolated in large amounts by simple methods, making them cost-effective vaccines. The first aim of this project is to clone each of the identified fimbrial gene clusters and to express the fimbriae on the surface of *E. coli*. Second, we will determine whether any of these fimbriae mediate *E. coli* binding to relevant epithelial cells or cells of the innate immune system. Third, in vivo expression of fimbriae will be determined by screening for antibodies in challenged mice. The importance of the fimbriae in pathogenesis will be studied with mutants. Fourth, mice will be immunized parenterally with fimbriae, and either orally or intra-nasally with an attenuated *Salmonella* vaccine strain expressing *Y. pestis* fimbriae. Systemic and mucosal humoral immune responses and the elicitation of anti-adhesive antibodies will be investigated. Challenge experiments will be undertaken to study immuno-protection by these vaccines.

**Grant:** 1R21AI053399-01A1  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** KAWULA, THOMAS H PHD  
**Title:** Molecular Basis of Francisella Virulence and Immunity  
**Institution:** UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC  
HILL  
**Project Period:** 2003/12/15-2005/11/30

DESCRIPTION (provided by applicant): *Francisella tularensis* is the etiologic agent of tularemia. This gram negative bacterium is a potential agent of bioterrorism because of its high mortality, extremely low infective dose, ability to survive for long periods of time outside of an animal host, and numerous transmission mechanisms including inhalation, ingestion, skin contact, mucous membrane contact, and insect vectors. Little is known about the mechanisms of *F. tularensis* pathogenesis and host resistance. *F. tularensis* will survive within macrophages, however macrophage-like cells infected with these organisms become apoptotic within 24 hours following inoculation. The first aim of this proposal is to examine the mechanism(s) by which *F. tularensis* provoke host cell death in macrophages. *F. tularensis* transposon insertion mutants that fail to induce macrophage apoptosis will be isolated and the mutations characterized to define bacterial products responsible for promoting host cell death. These mutant strains will be tested for potential attenuation in a mouse model of tularemia. The mechanism of apoptosis induction by *F. tularensis* will be determined using mouse gene chips possessing known pro- and anti- apoptotic gene sequences to compare macrophage mRNA levels from control and *F. tularensis* infected cells. The second aim is to isolate *Francisella* specific CD4 T cell hybrids and determine the differences in the epitopes recognized in C57Bl/6 mice infected with live or heat killed bacteria. *E. coli* clones expressing the apoptosis inducing genes identified in the first aim will be created and used to isolate specific CD4 T cell hybrids. The ultimate aim will be to create T cell clones of defined cytokine secretion (Th1 or Th2) (by using the defined peptide epitopes) and determine the effect of the clones on pathogenesis and protection. These aims are directed towards examining the molecular basis for *F. tularensis* pathogenesis, combined with an approach to elucidate potential immune response targets that could lead to improved prophylaxis against tularemia.

**Grant:** 1R21AI053407-01A1  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** BOHM, A ANDREW BS  
**Title:** Molecular Basis for Inhibition of Edema Factor  
**Institution:** TUFTS UNIVERSITY BOSTON BOSTON, MA  
**Project Period:** 2004/03/01-2006/02/28

DESCRIPTION (provided by applicant): The objective of this project is to find small molecule inhibitors of the anthrax protein Edema Factor (EF). This toxin is secreted by *Bacillus anthracis* in a catalytically inactive state. When the toxin is transported into the cellular cytoplasm of anthrax victims by the anthrax-derived transporter, Protective Antigen, it forms a complex with calmodulin (CAM) - the key intracellular calcium-binding protein in vertebrates. This association activates EF, and converts it into an adenylyl cyclase with 1000 times greater catalytic activity than the victim's own cyclic AMP-producing enzymes. Conventional anthrax therapies (which target the anthrax bacterium, not the anthrax toxins) are highly effective, but are insufficient to save all anthrax victims. EF inhibitors represent a completely distinct, and wholly complementary approach to combating anthrax. Such inhibitors will, by treating the downstream effects of the disease, help restore cellular equilibrium, and are likely to slow the course disease. By allowing more time for conventional treatments and the immune system to work, these inhibitors may significantly increase anthrax survival rates. We have solved crystal structures of EF (the inactive state) and the EF/CaM complex (the active state). Using these structures as a guide, and in close consultation with pharmaceutical researchers, we will use structure-based, computational drug discovery methods to identify small molecules representing two distinct types of EF inhibitors; Type A, inhibitors that occlude the active site, and Type B, those that maintain EF in its CAM-free, catalytically inactive state. We already have assays for inhibitors of Type A. We will complete the development of a simple assay for inhibitors of Type B, and then apply these assays to the top approximately 1000 compounds suggested by our computational drug screens. We will also determine the crystal structures of EF in complex with those small molecules identified by this screening procedure, so that the pharmaceutical properties of these inhibitors may be improved through rational drug design methods.

**Grant:** 1R21AI054473-01A2  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** MALKE, HORST PHD  
**Title:** Response of Streptococcus pyogenes to Nutritional Stress  
**Institution:** UNIVERSITY OF OKLAHOMA HLTH SCIENCES OKLAHOMA CITY, OK  
CTR  
**Project Period:** 2004/09/15-2006/08/31

DESCRIPTION (provided by applicant): Streptococcus pyogenes (group A streptococcus, GAS) causes various infections of humans including the skin, throat, deep tissue, and even the bloodstream. Although these sites are rich in peptides and proteins, free amino acids, which are essential for this polyauxotrophic organism, are not abundant. The molecular details of the adaptive response to such natural environments are incompletely explored. This response needs to be understood, therefore, at the level of a regulatory network that links basic metabolic processes to virulence gene expression and enables the organism in a dynamic way to take advantage of protein-rich host environments. Based on the advances made in the genomics of GAS in recent years, this study proposes to (i) determine the breadth of the amino acid starvation response of GAS on a genome-wide scale, by global transcription profiling using DNA microarray technology. This approach will allow description of the amino acid starvation response of stringent (RelA+) and relaxed strains (RelA-) in laboratory media as well as in conditions that mimic or constitute in-vivo situations at a comprehensive level. To link the stringent response to the activity of CodY, a pleiotropic transcriptional repressor that senses the nutritional state of the cell, this study proposes to (ii) identify the target genes of CodY and their transcription pattern as a function of the relA-determined guanosine polyphosphate level. While this approach will enable the response to nutritional stress to be understood at the level of transcriptional regulation of the responsive genes, the third aim of this study proposes to (iii) determine global mRNA degradation patterns at the sub-genic, genic and operonic levels and explore molecular mechanisms involved in the alteration of mRNA half-lives. This will provide information about how transcript stability is influenced by environmental conditions. Taken together, this research should result in a comprehensive network of adaptive responses to a key environmental condition that GAS may encounter in association with their host. This knowledge will help understand pathogenetic mechanisms and may even lead to new approaches for treatment or prevention of streptococcal infections.

**Grant:** 1R21AI055660-01A1  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** SULAKVELIDZE, ALEXANDER PHD  
**Title:** Genetic clustering and virulence of *Y. pestis* strains  
**Institution:** UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD  
SCHOOL  
**Project Period:** 2004/09/30-2006/08/31

DESCRIPTION (provided by applicant): *Y. pestis*, the etiologic agent of plague, was introduced into the United States at the turn of the 20th century; therefore, the genetic and phenotypic diversity of American *Y. pestis* isolates is relatively limited. In contrast, *Y. pestis* has been endemic in many countries of the former Soviet Union (FSU) for many centuries and numerous *Y. pestis*-related publications in the FSU's literature strongly suggest that the FSU's *Y. pestis* strains are genetically more diverse than are American *Y. pestis* isolates. Understanding the genetic and virulence trait differences among strains will be of critical importance during the development of advanced tools and strategies to deal with possible *Y. pestis*-related bioterrorism attacks in the USA and elsewhere. Therefore, we propose to begin a comprehensive characterization of *Y. pestis* strains from several endemic loci of plague in the FSU's Caucasus region, and to begin delineating the critical differences between the genetic make-up and virulence traits of *Y. pestis* strains from the FSU and elsewhere. Three specific aims will be addressed: (1) Characterize the genomic variability of *Y. pestis* strains from the FSU, and perform comparative MLST, PFGE, and MLVA analyses for *Y. pestis* fingerprinting, (2) Characterize the plasmid compositions of the FSU's *Y. pestis* isolates, and perform nucleotide sequence-based analyses of selected plasmid- and chromosomally-encoded virulence genes, and (3) Begin elucidating the prevalence and the expression patterns of major virulence genes in the FSU's *Y. pestis* isolates, using microarrays and RT PCR. The R21 grant mechanism is ideally suited for supporting our proposed studies, and it will help us to generate important information/initial data concerning the genetic make-up and gene expression patterns of *Y. pestis* isolates previously unavailable to investigators outside the FSU, and for which very little information is currently available. The generated data will also play a critical role in our designing appropriate future studies, and in expanding our *Y. pestis* research program at the University of Maryland School of Medicine.

**Grant:** 1R21AI055718-01A1  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** PICKETT, CAROL L PHD  
**Title:** C. jejuni CDT Subunit Interactions  
**Institution:** UNIVERSITY OF KENTUCKY LEXINGTON, KY  
**Project Period:** 2004/09/30-2005/08/31

DESCRIPTION (provided by applicant): *Campylobacter jejuni* is currently one of the most common bacterial causes of diarrheal disease in humans in the United States. It has also been strongly implicated as one of the most common infections to precede the development of Guillian-Barre's syndrome, an autoimmune disease. The study of *C. jejuni* pathogenesis has identified few potential virulence factors. One of these is the exotoxin, cytolethal distending toxin, (CDT). Recent work has identified 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 via direct DNA damage and invocation of the DNA damage checkpoint pathway. However, little is known about the specific interactions of the CDT subunits, CdtA, CdtB, and CdtC, with sensitive cells. Nor is much known about CDT subunit interactions within the CDT holotoxin. This information will be crucial for gaining an understanding of how to best develop vaccine strategies and interventions in diseases caused by bacteria that produce CDT. The broad, long-term goals of this proposal are to understand how CDT interacts with sensitive cells. In particular, we will determine the nature of the Cdt subunit interactions with the surface of sensitive cells, and explore the nature and extent to which the Cdt subunits interact with each other. These studies are central to our understanding of how CDT affects mammalian cells. The specific aims of the proposal are to test the hypothesis that the individual Cdt proteins have specific capabilities involved in recognizing a specific mammalian cell surface receptor, and in interacting with each other to form an effective holotoxin. In Aim 1, a variety of mutations will be made in each of the Cdt subunits. The effects of the mutations on toxicity, holotoxin assembly, and cell binding will be tested. In Aim 2, two different approaches will be used to identify areas within the Cdt proteins that are responsible for mediating the association of the subunits into holotoxin. In Aim 3, we propose to characterize and identify the CDT cell surface receptor. These studies should substantially increase our knowledge of CDT biology and allow for the development of new treatment and prevention strategies.

**Grant:** 1R21AI055963-01A1  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** KUDVA, INDIRA T PHD  
**Title:** E. coli O157:H7 genes induced during human infection  
**Institution:** MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA  
**Project Period:** 2004/04/01-2006/03/31

**DESCRIPTION** (provided by applicant): Microbial genes expressed exclusively during infection confer unique attributes to the organism. They are usually induced in response to specific signals encountered within the host and may encode factors that help a pathogen adapt to the host environment, establish itself in its niche, and cause disease. Consequently, such virulence genes are suitable targets for the development of diagnostic assays, prophylactic and therapeutic options against disease caused by that pathogen. Here, we propose the identification of *Escherichia coli* O157:H7 (O157) genes expressed only during human infection, using a recently described novel technique termed In Vivo-Induced Antigen Technology (IVIAT). This technique involves the sequential adsorption of convalescent sera from a patient who had hemolytic uremic syndrome (HUS) against whole cells, cell-lysates, and heat-denatured cell-lysates of the cognate pathogen grown under standard laboratory conditions. The adsorption process selectively removes antibodies against antigens expressed during in vitro growth, and enriches for those antibodies against in vivo-expressed antigens. Here, we will pool convalescent sera from three patients who recovered from HUS, adsorb them as described above against whole cells, cell lysates and heat-denatured cell lysates of three O157 strains isolated from the same patients who were the source of convalescent sera. The adsorbed convalescent serum pool will then be used to screen three individual O157 DNA expression libraries made from genomic DNA of the O157 strain isolated from the each of the above three patients. The genes contained in reactive clones will be identified by BLAST, against the genomes of O157 EDL 933 and Sakai strains, and other DNA sequences available in the National Center for Biotechnology Information (NCBI) non-redundant database and The Institute for Genomic Research (TIGR) database. Because of time and budgetary constraints, we will focus on only two or three of the IVIAT antigens. Previously unidentified antigens encoded by genes located on genomic sequences unique to O157 that are not transcribed during in vitro growth, will be purified via nickel affinity chromatography. Antibody responses against such antigens will be quantified in individual convalescent phase serum from the three patients described above. Ideal antigens for further study are those that are the target of robust antibody responses across all three patients. Such antigens (pending further studies) may help in the development of prophylactic options and could potentially serve as markers of ongoing or recent O157 disease in stool specimens. Broad, robust antibody responses against such antigens, could also serve as the basis for the development of serological diagnostic assays for recent O157 infection.

**Grant:** 1R21AI056048-01A1  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** PRITCHARD, DAVID G PHD  
**Title:** B. anthracis Peptidoglycan Deacetylase as a Drug Target  
**Institution:** UNIVERSITY OF ALABAMA AT BIRMINGHAM BIRMINGHAM, AL  
**Project Period:** 2004/07/01-2006/06/30

**DESCRIPTION** (provided by applicant): A high proportion of the acetamido sugar residues in the peptidoglycan layer of *B. anthracis* vegetative cells are de-N-acetylated by the action of one or more specific peptidoglycan deacetylase(s). This modification has the effect of making the bacterial cell wall resistant to digestion by lysozyme, a ubiquitous enzyme in human secretions, blood and tissues. The feasibility of using the peptidoglycan deacetylase of *B. anthracis* as a new drug target is assessed in this R21 application. A specific inhibitor of the enzyme could render the bacteria unable to protect their cell walls from host lysozyme and they would then be rapidly killed. Twelve candidate peptidoglycan deacetylase genes have been tentatively identified in the genome of *B. anthracis*. The first specific aim is to screen expressed candidate deacetylases for enzyme activity. To facilitate development of suitable analytical methods and biochemical procedures for use with the *B. anthracis* deacetylase, the only confirmed peptidoglycan deacetylase gene, the *pgdA* gene from *S. pneumoniae*, will be cloned first and expressed. Important properties of the enzymes will be determined, including their pH optima, metal ion requirements, if any, and the effect of reducing agents on enzyme activity. In addition, amino acid residues critical for enzymatic activity will be identified by means of site-directed mutagenesis of highly conserved regions of the enzymes. The second specific aim is to mutationally inactivate candidate deacetylase genes in *B. anthracis* and then assess the effects of the mutations on peptidoglycan deacetylation and sensitivity to lysozyme. In the unlikely event that none of the candidate genes encode the active deacetylase, the gene will be identified using a transposon mutagenesis, procedure. The third specific aim is to determine if deacetylase-deficient mutants are no longer virulent in a mouse model of *B. anthracis* infection. Virulence of wild type and mutant (i.e., peptidoglycan deacetylase-deficient) spores of the Sterne strain of *B. anthracis* will be compared using A/J and BALB/c mice, which are differentially sensitive to inoculation of Sterne spores. Mice will be challenged by three different routes, subcutaneous, intranasal, and intratracheal inoculation of spores. Success of this project may lead to the development of another valuable means of treating infections caused by other Gram-positive bacterial pathogens, in addition to *B. anthracis*, by inhibiting the deacetylases which specifically render their cell wall peptidoglycans resistant to the action of lysozyme.



**Grant:** 1R21AI056101-01A1  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** ABRAHAM, SOMAN N PHD  
**Title:** Proteome Mining in Caveolae of the Bladder Epithelium  
**Institution:** DUKE UNIVERSITY DURHAM, NC  
**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (provided by applicant): Urinary Tract Infections (UTIs) are one of the most common bacterial infections in man, and by far the single most common causative agent of UTIs is *Escherichia coli*. A critical aspect of bladder infection is bacterial invasion of the bladder epithelium. Recently, we discovered that invasion of human bladder epithelium and subsequent bacterial transcytosis was critically mediated by distinct plasmalemmal cellular entities called caveolae. Type 1 fimbriated uropathogenic *E.coli* were discovered to invade bladder cells by binding components of caveolae. *E.coli* invading via caveolae are encased in caveolar membranes and transcytosed without loss of viability. These observations point to caveolae as important portals for entry and infection. Very little is currently known of the caveolar structure within bladder cells, and how it is co-opted by pathogens for entry. It is also not known what keeps *E.coli* encased in caveolar proteins from fusing with lysosomes following entry. Determining the protein composition of caveolae in bladder epithelial cells before and after bacterial activation could derive valuable information regarding these issues. Here we propose to: (i) Use proteome mining approaches to identify protein composition of caveolae found in human bladder epithelial cells before and after interaction with *E.coli*. Our focus will be on molecules functioning as (a), receptors (b), signaling molecules and (c), mediators of cytoskeletal rearrangement and trafficking. (ii), Deduce the functions of selected caveolar proteins by specifically suppressing gene expression and examining its effects on the binding, invasion and/or intracellular trafficking activities of the *E.coli*. These proposed studies involve the utilization of exploratory technology (proteomics), which will complement other NIH funded research currently being undertaken.

**Grant:** 1R21AI056148-01A1  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** XU, JOHN L PHD  
**Title:** The Role of Host B Lymphocytes in Yersinia Pathogenesis  
**Institution:** UNIVERSITY OF ILLINOIS URBANA- CHAMPAIGN, IL  
CHAMPAIGN  
**Project Period:** 2004/09/30-2005/09/29

DESCRIPTION (provided by applicant): Studies of pathogenic Yersinia species over the last several decades yield important insights into the molecular mechanisms of bacterial virulence. However, our understanding of the immunology of Yersinia infection remains relatively primitive. Previous observations demonstrate that a specific Th1 response is critical for the clearance of Yersinia infection in mice, but the role of B cells in host defense against Yersinia has not been well studied. The broad, long-term objective of our research is to use a variety of mouse models to gain a detailed understanding of host factors that are important in mediating resistance or susceptibility to Yersinia and ultimately to use this knowledge to develop immune therapies for human plague. The focus of this project is to establish a role for host B cells in Yersinia pathogenesis by analyzing immune response to Yersinia infection in wild type, B cell-deficient (BCR-/-), and RAG-1-/- mice. We found that animals devoid of B cells exhibit enhanced early resistance to systemically administered Yersinia. However, BCR-/- mice that have survived an initial low-dose infection become only slightly more resistant than naive BCR-/- animals to re-challenge by a high dose of Yersinia. These observations suggest that B cells inhibit innate immune response to Yersinia but they are also involved in either the generation of effector T cells, or the maintenance of memory T cells, or both. Our goals are to elucidate the molecular and cellular mechanisms by which B cells modulate both innate immunity and primary and memory T-cell response during Yersinia infection. To achieve these goals, the pattern of cytokine production by B cells in response to Yersinia and Yersinia-secreted products will be analyzed, and the role of cytokines such as IL-10, IL-6, and TGF-beta in B cell-mediated inhibition of innate immunity to Yersinia infection will be examined. In addition, BCR-/- mice that lack certain subset of T cells will be generated and analyzed to determine whether BCR-/- mice are defective in T-cell response during Yersinia infection and which subset of T-cell response is defective. IgHEL BCR-/- mice will also be generated, and these animals can produce only hen egg lysozyme-specific B cells. Analysis of Yersinia infection in IgHEL BCR-/- mice will determine whether Yersinia-nonspecific B cells can correct the defective T-cell response observed in BCR-/- mice and whether cognate antigen presentation is required for B-cell induction of Th2 response. Furthermore, BCR-/- and IgHEL BCR-/- mice that have survived a primary infection will be re-challenged with a high dose of Yersinia and T-cell response will be analyzed to determine whether B cells and antibodies play a role in the maintenance of memory T cells. A detailed understanding of how Yersinia infection is cleared and how immunological memory is maintained will lead to informed decision on how best to design an anti-plague therapy.

**Grant:** 1R21AI057450-01A1  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** ZHONG, GUANGMING PHD  
**Title:** Chlamydial Manipulation of Host Apoptosis  
**Institution:** UNIVERSITY OF TEXAS HLTH SCI CTR SAN SAN ANTONIO, TX  
ANT  
**Project Period:** 2004/07/01-2005/06/30

Mechanisms of Chlamydial Manipulation of Host Cell Apoptosis Chlamydial infection in humans imposes a major health problem in both developing and developed nations. Urogenital tract infection with *C. trachomatis* species is a leading cause of sexually transmitted bacterial diseases and is also linked to certain type of cervical carcinoma while respiratory infection with *C. pneumoniae* species is associated with atherosclerosis, a major vascular condition for cardio-cerebral fatality. Although the species *C. psittaci* is primarily an animal pathogen, humans are also susceptible to *C. psittaci* infection, developing life-threatening pneumonia. Since humans can acquire infection via aerosolized animal feces that are contaminated with *C. psittaci* organisms, CDC has once listed *C. psittaci* as a category B agent for biodefense. These chlamydia-induced or -associated pathologies are largely due to chlamydial ability to either productively replicate or to achieve a long-term persistence within a cytoplasmic vacuole of eukaryotic cells, which are aided by the chlamydial unique intracellular biphasic life cycle and the chlamydial ability to evade host defense. The current proposal is designed to understand how chlamydia evades a very important host defense effector mechanism---apoptosis. We have previously demonstrated that chlamydia possesses a potent antiapoptotic activity, which may contribute to the chlamydial ability to survive in the infected hosts for long periods of time. By identifying the molecule(s) responsible for the chlamydial antiapoptotic activity and understanding how the antiapoptotic molecules work as proposed in the current project, we may be able to develop reagents/approaches for blocking the chlamydial antiapoptotic activity and preventing chlamydia-induced pathologies.

**Grant:** 1R21AI057579-01A1  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** WEINRAUCH, YVETTE Z PHD  
**Title:** Interaction of Enteroinvasive Pathogens with Neutrophils  
**Institution:** NEW YORK UNIVERSITY SCHOOL OF MEDICINE NEW YORK, NY  
**Project Period:** 2004/06/01-2006/05/31

DESCRIPTION (provided by applicant): Shigella, Salmonella and Yersinia spp. infect millions of people worldwide, these related pathogens cause different diseases, many of which can be lethal. Neutrophils play a central role in host defense against invading organisms. Within hours, activated neutrophils migrate to the site of infection where they deploy their granule associated anti-microbial arsenal. We recently identified a key host defense, granule protein; neutrophil elastase (NE) that rapidly and specifically destroys virulence factors of Shigella, Salmonella and Yersinia. It is unclear how NE recognizes and interacts with pathogenic bacteria. We hypothesize that the exposure of surface-bound granule proteins, including NE, of activated neutrophils to bacterial virulence factors is a critical aspect of their defense function and propose the following aims: (1) To identify bioactive NE on the surface of activated neutrophils and determine whether bacterial virulence factors are targeted at the neutrophil surface. NE and other neutral proteases associate with the membrane of activated neutrophils. Since the biological consequences of the interaction of bacterial virulence factors with surface-bound granule proteins of activated neutrophils are unknown we will examine the role of bioactive NE and other proteases on intact neutrophils in the degradation of virulence factors. (2a) Identification of pathogen specific neutrophil granule proteins by "affinity" purification with target bacteria. Role of NE. We have previously observed that the association of NE to the outer envelope of Shigella was more effective with bacteria treated with a crude lysate of neutrophils than with equivalent concentrations of purified NE (unpublished). Based on these observations we predict that targeting of virulence factors by NE is augmented by the preferential binding of granule proteins to Lipopolysaccharide (LPS) of intact bacteria. (2b) To examine the role of LPS specific granule proteins BPI and hCAP18 on NE recruitment and specificity. Specific neutrophil granule proteins such as Bactericidal Permeability Increasing protein (BPI) and hCAP18 have a high affinity for the outer envelope of Gram-negative bacteria. We have previously observed (unpublished) increased binding of purified NE to Shigella in the presence of hCAP18 but not in its absence. We propose that these proteins could potentially "recruit" NE to the bacterial envelope resulting in increased NE specificity.

**Grant:** 1R21AI057733-01A1  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** MCCLELLAND, MICHAEL PHD MOLECULAR GENETICS  
**Title:** Salmonella Gene Expression in Complex Environments  
**Institution:** SIDNEY KIMMEL CANCER CENTER SAN DIEGO, CA  
**Project Period:** 2004/09/30-2005/09/29

DESCRIPTION (provided by applicant): Salmonella causes tens of millions of infections in humans each year and it has been used as a bioterror weapon. This organism must contend with a highly complex gut microbiota in order to infect the host and the characteristics of the microbiota are known to affect the success of this infection process. Indeed, infectivity by Salmonella is reduced by 100,000-fold in the presence of normal gut flora. What are the genetic processes that underlie the interaction of a pathogen with commensal or symbiotic microbiota? These are difficult environments to investigate without reporter methods. Two methods, which could be used for studying pathogens in a population of other organisms, will be compared and contrasted using Salmonella as a model. In each of these methods, all the genes or promoters in a Salmonella genome will be tagged, en mass, with reporter systems. The mixture of bacteria, each individual carrying a single tag, will then be introduced into three groups of mice: abiotic, gnotobiotic, and normal. After growth in these environments, changes in the distribution of all the tags in Salmonella genes or promoters will be monitored on a Salmonella-specific microarray that we have built. The methods to be tested involve (i), selection for genes that are required in an environment by transposon tagging of the genome, and (ii), identification of genes that are induced, but not necessarily required, in an environment by screening for expression of a promoterless fluorescent marker in a promoter plasmid library. Tagging of genes and subsequent analysis by microarrays should allow monitoring of gene expression in complex environments that would be difficult or impossible to monitor by other methods. The methods may reveal new pathways that will lead to a better understanding of interactions with the microbiota, as well as revealing potential new vulnerabilities that can be exploited to fight these pathogens. As a class, these methods should be applicable to studying gene expression in any bacterial or eukaryotic microbial community.

**Grant:** 1R21AI057755-01A1  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** BENSON, ANDREW K PHD  
**Title:** Genome biology of *Francisella tularensis* populations  
**Institution:** UNIVERSITY OF NEBRASKA LINCOLN LINCOLN, NE  
**Project Period:** 2004/06/01-2006/05/31

DESCRIPTION (provided by applicant): *Francisella tularensis* is a highly infectious, risk-group A select bacterial agent that has the potential to be weaponized. It is the cause of tularemia. The applicants' long-term goal is to understand the pathways of genes and gene products that drive unique virulence and transmissibility traits of the four subspecies of *F. tularensis*. The objective of this R21 application is to identify genes that are responsible for virulence and transmissibility of the most virulent subspecies of this bacterium. The central hypothesis is that increased virulence and transmissibility are attributable to genetic differences. This hypothesis will be tested by pursuing three specific aims: 1) Design and characterize a reference strain collection representing both *F. tularensis* subsp. *tularensis* and subsp. *holarctica* with temporal and spatial representation; 2) Fabricate representative reference microarrays of the *tularensis* and *holarctica* subsp. and systematically probe genome diversity of the strain collection using arrays; and 3) Use paired-end sequence mapping to complement data from the microarray-based studies. The principal approaches and methods to be used include alternative strategies and tactics to systematically catalogue subspecies-specific genome segments. This will be accomplished using DNA microarray analyses (arrays derived from shotgun libraries prepared from the representative subsp. *tularensis* and *holarctica*) and a novel algorithm for data sorting followed by DNA sequence analyses and confirmation by Southern blotting and PCR. Second, a narrow (10Kb)-size library from a representative *holarctica* strain will be used to generate paired-end sequence reads, after which the list will be mapped to the subsp. *tularensis* genome sequence. This will allow length differences that are subspecies-specific to be identified. The outcomes are collectively significant, because they are expected to provide strong preliminary evidence that strain differences in virulence and transmissibility are attributable to genomic differences. Such data are expected to become the foundation for definitive R01-level investigations involving the functional analyses of candidate genes. These studies will significantly increase our understanding of the pathobiology of this organism, provide candidate gene products for vaccine and therapeutics, and provide loci for development of improved diagnostic and forensic markers.

**Grant:** 1R21AI057778-01A1  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** DIAKONOVA, MARIA PHD  
**Title:** Role of Adapter Protein in Infectious Diseases  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2004/06/01-2006/05/31

DESCRIPTION (provided by applicant): *Listeria monocytogenes* is a food-borne pathogen that can cause meningitis, meningoencephalitis, septicemias, abortions and, in some cases, gastroenteritis. The overall mortality rate is >20% and fetal or neonatal infection with *Listeria* has an even higher mortality. *Listeria* invades a broad range of cell types. Intracellular *Listeria* replicates in the cytoplasm of host cells and induces the polymerization of host actin filaments ("actin tails") at the bacteria surface using bacterial protein ActA. Actin-based motility allows *Listeria* to spread from cell to cell without leaving the protective intracellular niche, and is essential for pathogenesis. However, the mechanism underlying *Listeria* motility and spreading remains elusive. The adapter protein SH2- Bbeta regulates cell motility. I have implicated SH2- Bbeta in the motility of *Listeria*. Preliminary data revealed that *Listeria* in cells overexpressing wild type SH2- Bbeta demonstrates increased velocity (225% of control) while expression of SH2 domain-deficient mutants of SH2- Bbeta in host cells inhibits *Listeria* movement (by approximately 60%). In a cell-free system using *Xenopus* oocyte extracts and purified GST-SH2-Bbeta, SH2-Ba increased the velocity of *Listeria* by 140% of control. I have shown that SH2- Bbeta binds to VASP/profilin two proteins that have been shown to participate in actin-dependent *Listeria* motility. This application tests the hypothesis that SH2- Bbeta promotes *Listeria* infection by stimulating actin-based motility. The first aim will determine whether VASP/ profilin directly bind(s) to SH2- Bbeta. The second aim will determine whether SH2- Bbeta interaction with VASP/profilin is required for *Listeria* motility. The third aim will test whether SH2- Ba is required for spreading of *Listeria* infection. The fourth aim will examine whether SH2- Bbeta is required for the virulence of *Listeria*. In addition to providing insight into the molecular mechanism by which SH2- Bbeta contributes to *Listeria* motility, the results of the application studies will increase our understanding of the fundamental mechanism by which *Listeria* spreads. These studies designed to identify new proteins and signaling pathways involved in *Listeria* motility may identify new therapeutic targets for preventing the rapid distribution of *Listeria* infection and thereby protect people from listeriosis.

**Grant:** 1R21AI057781-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** KAPLAN, GILLA PHD  
MICROBIOLOGY:BACTERIOLOG  
Y  
**Title:** Cytokines in Pathogenesis of Anthrax Infection  
**Institution:** PUBLIC HEALTH RESEARCH INSTITUTE NEWARK, NJ  
**Project Period:** 2004/04/15-2006/03/31

DESCRIPTION (provided by applicant): Death from systemic anthrax in humans results from massive inflammation, multi-organ failure and shock caused by harmful levels of *Bacillus anthracis* exotoxins. Early antibiotic treatment can improve survival by eliminating infectious organisms, however, most victims succumb because antibiotics are administered too late in the course of anthrax infection when toxins have already reached critically high levels. It is our hypothesis that, in addition to the direct effects of the anthrax toxins, specific proinflammatory cytokines produced in response to infection with *B. anthracis* contribute significantly to the pathogenesis of systemic disease. In our proposed study, we will define the proinflammatory cytokine cascade in *B. anthracis*-infected monocytes in vitro, in mice infected by inhalation of *B. anthracis* and in rabbits with anthrax meningitis. We will investigate whether inhibition of the production of specific proinflammatory cytokines improves outcome in these animal models of *B. anthracis* infection and treatment. In particular, we will study the effects of antibiotics combined with immunomodulatory drugs (thalidomide analogues) on the pathogenesis of anthrax meningitis, reported in 50% of human cases of systemic anthrax and associated with long term CNS damage and impaired cognitive functions in survivors of this condition. We have access to suitable BL3 facilities, experience in working with virulent clinical isolates of *Mycobacterium tuberculosis* both in vitro (in cultured fresh human monocytes) and in vivo (in mouse and rabbit models of infection), and access to novel immunomodulatory drugs (thalidomide analogues). Thus we are in a unique position to conduct the proposed studies. We believe that an improved understanding of the cytokine cascade which may be exacerbated by products released from bacilli killed by antibiotic treatment, and better insight into the contribution of cytokines to the pathology of anthrax will enable the design of superior treatment regimens as clinical countermeasures using antibiotics supplemented by selected immunomodulatory drugs.



**Grant:** 1R21AI057875-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** HE, YONGQUN PHD  
**Title:** GENE EXPRESSION IN BRUCELLA-INFECTED MACROPHAGES  
**Institution:** VIRGINIA POLYTECHNIC INST AND ST UNIV BLACKSBURG, VA  
**Project Period:** 2004/02/15-2006/01/31

DESCRIPTION (provided by applicant): Brucella are intracellular facultative bacteria causing brucellosis in animals and humans. Four Brucella species are pathogenic to humans and classified as NIAID category B priority pathogens. However, no safe and effective Brucella vaccine is available for human use and the basic mechanisms by which Brucella successfully replicate intracellularly in host tissues remains unclear. B. melitensis, B. suis, and B. abortus are the most virulent to humans. The presence of the O-side chain in lipopolysaccharide (LPS) distinguishes smooth virulent Brucella strains from rough attenuated or avirulent strains. Brucella O-side chain has been shown to induce protective cell mediated immunity. Smooth Brucella infect and replicate within host macrophages whereas rough strains infect and replicate for a limited time. We propose to use high throughput DNA microarray technology to study differential and coordinated gene expression in the time course of murine macrophage responses to infection with smooth and rough Brucella strains. The role of Brucella O-side chain in stimulation of specific macrophage gene responses will be analyzed. The gene expression profiles detected by DNA microarray experiments will be confirmed by real-time RT-PCR. The proposed research will greatly help in understanding the genetic basis of host defenses against infection by virulent and attenuated Brucella strains and make possible development of novel strategies to treat and prevent infections.

**Grant:** 1R21AI057889-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** ZANETTI, MAURIZIO MD  
**Title:** Conformationally-Constrained PA Anthrax Vaccine  
**Institution:** UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA  
**Project Period:** 2004/09/30-2006/08/31

DESCRIPTION (provided by applicant): *Bacillus anthracis*, the agent that causes anthrax, has several characteristics that make it a formidable bioterrorist threat. Presently efforts to develop an effective anthrax vaccine can be categorized into the following groups: (1) Protein vaccines; (2) Live attenuated vaccines; (3) DNA and replicon vaccines; and (4) Identification of new antigens. The steps in host-cell intoxication have been recently clarified. An 83-kDa form of protective antigen (PA83) is secreted from rapidly growing *B. Anthracis* cells and binds via a 19aa solvent-exposed loop in domain 4 between strand 4A-4B of the protective antigen (PA) to a specific host cell surface receptor termed ATR. X-ray crystallography studies characterized this loop as having the structure of the complementarity-determining region (CDR) of an immunoglobulin (Ig). It is the goal of this application to develop and test as a proof-of-principle a series of Ig molecules expressing a conformationally-constrained 4B9-4B10 PA loop, antigenized antibodies. Furthermore, we will ascertain whether immunization with antibodies antigenized to express 4B9-4B 10 PA loop will result in the induction of site specific antibodies (i.e., directed at the 4B9-4B 10 PA loop) also able to neutralize the internalization of *B. anthracis* toxin and its cytopathicity. It is hoped that the idea and experiments proposed in this application can speed up the development of a safe and effective method to vaccinate against *B. anthracis*.

**Grant:** 1R21AI057940-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** NARASIMHAN, SUKANYA PHD  
**Title:** Tick gene expression- in the context of Lyme disease  
**Institution:** YALE UNIVERSITY NEW HAVEN, CT  
**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (provided by the applicant): This R21 proposal builds upon the observations made in the RO1 (A1032947-11) which indicates that the expression of TROSPA, a tick receptor for OspA, is up regulated upon infection by *Borrelia burgdorferi* (*B. burgdorferi*), the causative agent of Lyme disease. This presumably facilitates *Borrelia* colonization of the tick midgut. *B. burgdorferi* likely modulates the tick transcriptome to ensure its survival and transmission. This aspect of Lyme disease has not been investigated and could open novel venues to vector and disease control. The proposed project aims to broaden the scope of the RO1 and shift the focus beyond TROSPA and OspA. Using cDNA arrays the global changes that occur in the tick salivary gland transcriptome during the infection and transmission of *B. burgdorferi* will be defined. The functional significance of these gene products in enabling *B. burgdorferi* invasion of tick salivary glands and its subsequent transmission will be evaluated by specifically silencing the selected tick genes in vivo, using the RNA interference technology. The results of this project will provide insights into tick-*Borrelia* interactions and enable identification of novel tick -based targets for blocking transmission of Lyme disease. This study will also serve to develop the infrastructure and genetic tools essential for making rapid progress in the field of tick functional genomics. This investigation may therefore serve to pave the way for elucidation of mechanisms underlying transmission of other tick-borne pathogens.

**Grant:** 1R21AI057952-01A1  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** HIGH, KEVIN P MD  
**Title:** Brucella, Aging and Role of IL-17 in Host Defense  
**Institution:** WAKE FOREST UNIVERSITY HEALTH SCIENCES WISNTON-SALEM, NC  
**Project Period:** 2004/07/01-2006/06/30

DESCRIPTION (provided by applicant): Adults over the age of 65 comprise the fastest growing segment of the U.S. population. Aging increases susceptibility to most intracellular microbes (e.g. Mycobacterium, Salmonella, influenza and other viruses), likely due to waning immunity with advanced age termed 'immune senescence'. Immune senescence is characterized by impaired Th1 immunity, and efforts to reverse the responses that wane in immune senescence have been largely unsuccessful. However, recent data suggest augmenting immune responses that remain intact, even in far advanced age, may be a more achievable strategy to reduce the burden of infectious diseases in older adults. Brucella spp., important causes of disease in both human and animals, represent an exception to the rule that age increases the risk of infection due to intracellular pathogens. Scant published literature and our preliminary experiments suggest older adult mice and humans are no more susceptible to Brucella infection, and may in fact be less susceptible to this pathogen. In this proposal, we will use murine models to explore the immune mechanisms that remain intact or are enhanced with age, that allow efficient clearance of Brucella infection. Our preliminary data demonstrate marked increases in the poorly studied T cell cytokine IL-17 in response to whole Brucella organisms and specific Brucella antigens, particularly in older mice when compared to young adult mice. We suspect that the enhanced resistance of older adult mice may be due to IL-17, and the first aim of this proposal is to better define this association across the age spectrum. Very recent data have suggested IL-17 responses may be mediated by the antigen presenting cell-derived cytokine IL-23. This recently discovered feedback loop at the innate/adaptive interface may be an IL-12 independent mechanism to activate Th1 immune responses, a critical need to enhance resistance to intracellular pathogens in older adults. Initial investigations in this regard form the basis of our second aim. Finally, we will determine the clinical relevance of IL-17 in host defense by blocking the activity of this cytokine in murine models of brucellosis, and assessing the presence of IL-17 secreting memory T cells in humans with active or past Brucella infection. The data generated in this proposal will form the foundation of future R01 proposals aimed at enhancing immunity vs. intracellular pathogens in our aging population.

**Grant:** 1R21AI057974-01A1

**Program Director:** BAKER, PHILLIP J.

**Principal Investigator:** KHAN, SALEEM A. PHD  
BIOCHEMISTRY:BIOCHEMISTR  
Y-UNSPEC

**Title:** Plasmid pXO2 Replication in *Bacillus anthracis*

**Institution:** UNIVERSITY OF PITTSBURGH AT PITTSBURGH PITTSBURGH, PA

**Project Period:** 2004/07/01-2006/06/30

DESCRIPTION (provided by applicant): *Bacillus anthracis* is an important human pathogen and a potential biological weapon. Two large plasmids, pXO1 and pXO2 play a major role in the virulence of this organism. Very little is known about the molecular mechanisms involved in the replication and stability of the two virulence plasmids. Gene transfer can frequently occur between *B. anthracis* and closely related species such as *Bacillus cereus*, *Bacillus thuringiensis* and *Bacillus mycoides*, making it likely that the pXO1 and pXO2 plasmids could naturally transfer from *B. anthracis* into related species that are resistant to one or more antibiotics. Also, the possibility that bioterrorists may introduce the pXO1 and pXO2 plasmids into multiple drug resistant strains to generate "super bioterror agents" cannot be discounted. Given these possibilities, it is important to identify plasmid pXO2 (and pXO1)-specific drugs that could interfere with plasmid replication and can be used for the elimination of plasmids from *B. anthracis* and related organisms. The goal of this R21 proposal is to study the replication properties of the pXO2 plasmid of *B. anthracis*. The minimal replicon of pXO2 will be identified and the host range of the mini pXO2 plasmid studied by its ability to be established in *B. anthracis* and other Gram-positive bacteria such as *B. cereus*, *B. thuringiensis*, *Bacillus subtilis*, *Staphylococcus aureus*, *Clostridium perfringens* and *Streptococcus pneumoniae*. The role of the RepB protein of pXO2 in plasmid copy number control and stability will be investigated by estimating the copy number of mini pXO2 in different hosts and by measuring percent plasmid loss per generation during bacterial growth. The interaction between the RepS initiator protein and the origin of replication of pXO2 will be studied by electrophoretic mobility-shift assays and by DMS footprinting. Regions of pXO2 origin that interact with the RepS protein will be mutated and the ability of these mutants to support replication will be tested. A correlation between RepS-origin interaction and plasmid pXO2 replication will be established. We will also make cell-free extracts from *B. anthracis* and use these to study pXO2 replication in vitro. Our studies may reveal new molecular targets for therapeutics that affect plasmid replication and/or maintenance during infection.

**Grant:** 1R21AI058002-01  
**Program Director:** ZOU, LANLING  
**Principal Investigator:** HUANG, CHUN-MING PHD  
**Title:** Anthrax vaccination by targeting spore germination  
**Institution:** UNIVERSITY OF ALABAMA AT BIRMINGHAM BIRMINGHAM, AL  
**Project Period:** 2004/09/30-2006/08/31

DESCRIPTION (provided by applicant): Germination occurs as an upstream event during the life cycle of *Bacillus anthracis*. Germinating spores may be considered the weakest link during its life cycle because stopping their function would prevent further development of the disease. Therefore, the development of vaccines against specific proteins expressed during the germination stage of *Bacillus anthracis* may be an effective strategy to halt the growth of anthrax. Blocking the germination stage would prevent downstream events including the production of anthrax's three natural toxins, PA, LF, and EF, as well as any other foreign toxins that bioterrorists may insert into anthrax. We have recently characterized twenty-two germination-associated proteins from *Bacillus anthracis* by using proteomic techniques. Conventional proteomics studies proteins primarily using 2D electrophoresis and mass spectrometry. We have determined these germination proteins are differentially expressed from spore dormancy to germination. Eleven identified proteins have been identified. They include one secreted protein (Immune inhibitor A), four potential membrane-associated proteins (Camelysin, Alanine racemase, Larabinose transporter, and L-type calcium channel), two molecular chaperones (HSP60 and cpn60), two energy-related proteins (ATP synthase and Glyceraldehyde 3-phosphate dehydrogenase) and one novel anthrax specific protein. Immune inhibitor A and camelysin are virulent metalloproteinases and have been unreported in *Bacillus anthracis*. Dr. Tang, a Co-PI in this study, has well-established a noninvasive vaccination system based on vector based vaccines applied to the skin. That system will be used in this study to develop anthrax vaccines that will specifically target two of the germination-associated proteins: immune inhibitor A and camelysin. We will also compare current PA and LF targeted vaccines that have been constructed in our laboratory. In parallel, vaccines derived from immune inhibitor A and camelysin will be evaluated in vitro and in vivo to determine their efficacies in blocking spore germination and cytotoxicity to macrophage cells. This unprecedented study will elicit the pathological mechanisms and vaccination capabilities of immune inhibitor A and camelysin expressed in *Bacillus anthracis*.

**Grant:** 1R21AI058011-01  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** GREEN, G N BS  
**Title:** Intracellular Inhibitors of Botulinum Neurotoxins  
**Institution:** VANDERBILT UNIVERSITY NASHVILLE, TN  
**Project Period:** 2004/02/01-2006/01/31

DESCRIPTION (provided by applicant): The emerging bioterrorism threat has galvanized the need for rapidly effective treatments against botulism. This acute neuromuscular condition is produced by botulinum neurotoxin (BoNT), which ranks among the most deadly toxins known. The BoNT light chain (BoNT-LC) is a highly specific endopeptidase that inhibits neurotransmitter release from presynaptic membranes inside human neuronal cells. To meet the challenge posed by the rapid intracellular entry of BoNT, experiments are proposed to develop recombinant BoNT inhibitors that can be swiftly delivered to the neuronal-cell cytosol. In Aim 1, we will identify single-chain fragment variable antibodies that trap the LC of BoNT (serotype B) and prevent its functional maturation. Variants of this prototype inhibitor will be engineered that inactivate BoNT/B by blocking access to the catalytic site of BoNT/B-LC (Aims 2 & 3). We will enable the inhibitors to cross plasma membrane using a proprietary intracellular delivery system, thus generating "intracellular toxin traps" that bind to and inactivate BoNT within the intracellular compartment where the peptidase normally operates. These highly integrated studies will generate a new type of intracellular therapeutic and fill an existing gap in currently available measures to counteract the lethal effects of BoNT.

**Grant:** 1R21AI058123-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** VUORI, KRISTIINA MD  
**Title:** Mechanism of anti-phagocytosis by *Yersinia pestis*  
**Institution:** BURNHAM INSTITUTE LA JOLLA, CA  
**Project Period:** 2004/01/15-2005/12/31

DESCRIPTION (provided by applicant): *Yersinia pestis*, the causative agent of plague, is one of the most pathogenic bacteria known to mankind. Due to its high pathogenicity, *Yersinia* has been recognized as a potential weapon for bioterrorism. An aerosolized form of the bacteria in particular could cause a pneumonic infection, which could infect a large number of people very rapidly. While early antibiotic treatment is usually effective in the bubonic form of plague, the pneumonic plague is less responsive and often results in death. Additional modes of treatment would therefore be required to combat plague following exposure of a population to weaponized *Yersinia*. *Yersinia*'s high pathogenicity is due to its capability to evade the immune system. Thus, while most other bacteria are effectively ingested by integrin-mediated engulfment and destroyed by phagocytes, *Yersinia* blocks phagocytosis. The mechanism of "anti-phagocytosis" involves the virulence factor YopH, a highly effective tyrosine phosphatase. One crucial target for YopH is thought to be the docking protein p130cas(Cas), but the precise mechanism of its action remains unknown. In Aim 1, we will utilize our familiarity with integrin signaling and the Cas protein to examine the molecular mechanism antiphagocytosis. Our studies indicate that Cas is required for activation of the Rac GTPase in response to integrin ligation, and that this activation is essential for phagocytosis. Our data support the notion that the N-terminal domain of YopH binds to Cas, which correlates with YopH-mediated anti-phagocytosis. We will test the hypothesis that YopH functions by inhibiting a complex formation between Cas and its binding partner Crk and subsequent Rac activation. We will also test an alternative, although not mutually exclusive hypothesis that the YopH-Cas interaction results in targeting of YopH to the sites of phagocytosis, and in the subsequent inactivation of yet-to-be-identified target molecules. Substrate-trapping technology and proteomics approaches combined with functional assays will be used to identify and characterize these target proteins. Aim 2 will take advantage of the expertise of Dr. Maurizio Pellecchia, our collaborator, in NMR-based drug discovery and structural biology with YopH. Our goal is to develop YopH-specific small molecule inhibitors that block the function of the N-terminal domain of YopH. A combination of chemical library screening and NMR-based design will be used. Lead compounds will be evaluated for efficacy in the phagocytosis and signaling assays established in Aim 1 above. This application will combine complementary expertise of a cell biological and a structural biological laboratory to address a fundamentally important question in the *Yersinia* pathogenesis in a unique manner. We anticipate that these studies, which are supported by a significant amount of preliminary data, will allow us to better understand the mechanisms by which *Yersinia* block the antimicrobial function of phagocytes. We further expect that the inhibitors to be identified will be a valuable starting point for the further development of novel drugs that can be used to combat plague mortality.



**Grant:** 1R21AI058161-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** HOLLINGSWORTH, JOHN W MD BIOLOGY  
**Title:** Genetic susceptibility to F tularensis  
**Institution:** DUKE UNIVERSITY DURHAM, NC  
**Project Period:** 2004/09/30-2006/08/31

DESCRIPTION (provided by applicant): *Francisella tularensis* is the causative organism of the disease tularemia. The extreme pathogenic and infectious properties of this organism, as well as its ease of dissemination, make it a plausible candidate for use in a biological weapon. *F. tularensis* can be rendered even more dangerous by its aerosolization and by transformation with plasmids encoding resistance to antibiotics. It is therefore imperative to explore novel therapies for inhaled *F. tularensis* that do not rely on the use of these drugs. Developing such therapies would be facilitated by an improved understanding of the host genes participating in the clearance of *F. tularensis* from the lungs of experimental mice. Toward that end, I propose to use two parallel gene identification strategies: quantitative trait locus (QTL) mapping, and microarray-based gene expression studies. Individually, each of these approaches yields more candidate genes than can be feasibly pursued within the time limits of this proposal. To overcome this problem, I will reduce the candidate gene number to a manageable level by selecting only those genes that are contained within identified QTL and that are induced by *F. tularensis* infection. This comparatively small subset is likely to contain genes causally associated with susceptibility to *F. tularensis*. These genes will be tested for their biologic relevance by selectively suppressing them in cultured macrophages using short interference RNA (siRNA). Macrophages transfected with gene-specific, siRNA-expressing plasmids will be compared to their untransfected counterparts for their abilities to support proliferation of *F. tularensis* in vitro. Biologically validated genes emerging from these studies will represent novel molecular targets whose actions might be manipulated by therapies designed to improve the innate immune response to *F. tularensis*.

**Grant:** 1R21AI058699-01  
**Program Director:** NEAR, KAREN A.  
**Principal Investigator:** PINE, RICHARD PHD  
**Title:** HIV-1 and M. tuberculosis Co-infection Modeled In Vitro  
**Institution:** PUBLIC HEALTH RESEARCH INSTITUTE NEWARK, NJ  
**Project Period:** 2004/03/01-2006/02/28

DESCRIPTION (provided by applicant): The coincidence of AIDS and tuberculosis is such a major public health threat that it has been called the "cursed duet". We seek to learn how HIV-1 affects the pathogenicity of M. tuberculosis and vice versa. In regions where tuberculosis is endemic, co-infection mainly reflects re-activation of latent tuberculosis with the onset of AIDS. However, in some populations, infection by HIV-1 precedes acute exposure to M. tuberculosis. Moreover, active tuberculosis also might affect the course of initial HIV-1 infection. In vitro studies of co-infection have not compared order of infection. We will test the hypothesis that the order of infection will influence the outcome of co-infection. This can be done most effectively with a functional genomics approach that is not constrained by pre-judgement of what differences are likely. Functional genomic tests of global hypotheses also provide abundant data to generate new hypotheses that may lead in unexpected directions. For example, our functional genomic data from human macrophages infected by M. tuberculosis, revealed increased expression of the HIV-1 co-receptor CXCR4. That single observation suggested a novel and significant aspect of HIV-macrophage interaction during tuberculosis that we then tested in co-infection experiments. To date, neither infection of macrophages by HIV-1 nor co-infection has been studied with functional genomics. Our aim is to characterize differences in human macrophage gene expression profiles in cells coinfecting by HIV-1 and M. tuberculosis compared to cells that are uninfected or infected by only one pathogen, for two distinct co-infection scenarios modeled in vitro. We will infect first by HIV-1 and then by M. tuberculosis or vice versa. The gene expression profiles will be characterized using a spotted microarray of highly specific 60-base oligonucleotide probes for approximately 19,000 human genes. Replicate experiments will be performed so that statistically significant changes in gene expression can be determined, and these will be analyzed using algorithms for Boolean logic, clustering, and profiling. Based on bioinformatic analyses and on informed appreciation of individual changes in gene expression, we will develop new hypotheses. Moreover, our data will clearly show whether co-infected cells exhibit gene expression profiles that differ based on whether they were infected first by HIV-1 and then by M. tuberculosis or vice versa. Such differences are expected to provide a basis for addressing the course of disease in different groups of patients represented by the models.

**Grant:** 1R21AI058785-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** TONGE, PETER J PHD  
**Title:** Menaquinone Biosynthesis in *M. Tuberculosis*  
**Institution:** STATE UNIVERSITY NEW YORK STONY BROOK STONY BROOK, NY  
**Project Period:** 2004/06/15-2006/05/31

DESCRIPTION (provided by applicant): *Mycobacterium tuberculosis* is a major opportunistic pathogen in patients with HIV-AIDS. Current tuberculosis treatment regimes are severely hampered by the occurrence of multidrug resistant strains of *M. tuberculosis* and there is a critical need for the development of novel chemotherapeutics. In this proposal we initiate studies on the menaquinone biosynthesis pathway in *M. tuberculosis*. Menaquinone is the sole quinone in the mycobacterial electron transport chain and, since the pathway leading to the biosynthesis of menaquinone is absent in humans, the bacterial enzymes catalyzing the synthesis of menaquinone from chorismate are potential novel targets for drug discovery. The biosynthesis of menaquinone has been extensively studied in *E. coli* and *B. subtilis*. Homologs of all but one of the *E. coli* enzymes are present in *M. tuberculosis*. In Specific Aim 1 we will clone, express and purify all of the putative menaquinone biosynthetic enzymes from *M. tuberculosis*. We will then determine if the mycobacterial enzymes catalyze the reactions that have been predicted for them based on sequence homology. The availability of recombinant, active enzymes with known function is essential for the detailed enzymological studies that will form the basis of future efforts to rationally design compounds targeted at menaquinone biosynthesis in *M. tuberculosis*. In order to determine which menaquinone biosynthetic enzymes will be suitable for future inhibitor design, we need to know whether inhibition of a specific enzyme will impact the viability of the bacterium. In Specific Aim 2 we will use gene replacement methods to knockout individual menaquinone genes and determine the effect of the knockouts on bacterial survival. Knowledge of the phenotype caused by inactivation of a specific enzyme through knockout experiments will be an important reference point for future studies when the effect of specific enzyme inhibitors on the bacterium are evaluated.

**Grant:** 1R21AI059061-01  
**Program Director:** PETERS, N KENT  
**Principal Investigator:** BALABAN, NAOMI PHD  
**Title:** Quorum sensing and Staphylococcus aureus pathogenesis  
**Institution:** TUFTS UNIVERSITY BOSTON BOSOTN, MA  
**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (provided by applicant): This proposal is responsive to PA-03-080 on "Biodefense and emerging infectious disease research opportunities". Our aim is to better understand how virulence is regulated in Staphylococcus aureus and to further develop immunotherapy to staphylococcal infections. S. aureus cause food poisoning, sepsis, device (Biofilm) related infections, and multiple diseases like endocarditis and pneumonia. The numerous toxins produced by the bacteria are at the heart of these diseases and cause more than 50,000 deaths each year in the US alone. As food and device-associated infectious organism that can survive in multiple environments, S. aureus is a potential target for bioterrorism. Many of the infective strains are resistant to conventional antibiotics and alternative methods to prevent and treat such infections are mandatory. We discovered that the production of toxins is regulated by quorum sensing mechanisms, where proteins such as RNAIII activating protein (RAP) are secreted by the bacteria and induce virulence. RAP (native or recombinant) activates S. aureus pathogenesis through the histidine-phosphorylation of TRAP in a still unknown manner. TRAP has been shown to be membrane-associated, but it lacks a predicted transmembrane domain and a kinase domain typical of two component system sensors. Our first hypothesis is that TRAP is bound to the membrane through another protein and that this protein acts as the actual receptor to RAP. Studies proposed in Specific Aim 1 are focused on identifying this receptor. RAP has been shown to serve as an effective vaccine to prevent S. aureus infections. Our second hypothesis is that monoclonal anti RAP antibodies may be used for immunotherapy. This will be tested under Specific Aim 2, using a panel of clones already available. Such immune intervention would greatly benefit people suffering from acute drug resistant infections or surgery-related infections often associated with implantable medical devices.

**Grant:** 1R21AI059064-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** CHEN, WANGXUE PHD  
**Title:** Mouse model of oral infection with virulent Francisella  
**Institution:** NATIONAL RESEARCH COUNCIL OF CANADA OTTAWA, ON  
**Project Period:** 2004/08/01-2006/07/31

DESCRIPTION (provided by applicant): Francisella tularensis, the causative agent of tularemia, is classified as a Category A biological warfare agent by The Working Group on Civilian Biodefense because of its extreme infectivity, ease of dissemination, and substantial capacity to cause significant illness and death within a short period of time. Although aerosol-initiated F. tularensis infection is considered as the prime choice for a bioterrorist attack, deliberate contamination of drinking water and the food supply could be an alternative means to spread the infection. This approach would be especially attractive to non-state-supported individuals or groups of fanatical terrorists, who do not have the necessary equipment or technical expertise to generate an effective infectious aerosol. Most current research on F. tularensis has used a systemic infection model with the attenuated live vaccine strain (LVS) of F. tularensis. In contrast, very little is known about the pathogenesis of and immunity to virulent F. tularensis infections, especially following ingestion of the pathogen. Moreover, it is unknown whether or not the current live vaccine, F. tularensis LVS, is effective against oral infection with virulent F. tularensis in the event of food- and water- borne outbreaks because the mechanisms of host defense against type A F. tularensis infection initiated via different routes appear to be different. The primary goal of this R21 application in response to PA-03-080, "Biodefense and Emerging Infectious Diseases Research Opportunities", is to develop and characterize a mouse model of oral infection with type A F. tularensis. Specifically, we aim to: 1) characterize the immunopathogenesis of oral infection with type A F. tularensis in mice; 2) determine the susceptibility of immunocompromised hosts to oral infection with type A F. tularensis; and 3) determine the effectiveness of LVS vaccine against oral infection with virulent F. tularensis. The mouse model developed and characterized under this R21 proposal will be a useful tool for future studies on the immune mechanisms of host defense against food- and water- borne tularemia and for the development and evaluation of effective vaccines and immunotherapeutic agents against virulent F. tularensis infection, the long-term objective of our research project. Results from this proposal will also provide valuable preliminary but fundamental information on the immunopathogenesis of oral F. tularensis infection so that future hypothesis-driven studies with more focused research objectives can be designed and developed.

**Grant:** 1R21AI059095-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** BLANKE, STEVEN R PHD BIOCHEMISTRY  
**Title:** C. jejuni Cytolethal Distending Toxin Cell Interactions  
**Institution:** UNIVERSITY OF HOUSTON HOUSTON, TX  
**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (provided by applicant): *Campylobacter jejuni* is a food and water borne pathogen responsible for severe inflammatory diarrhea in humans. *C. jejuni* is a NIAID Category B Priority Pathogen because, in part, of the potential of this pathogen to be used as a biowarfare agent to contaminate food- and water supplies over large geographical areas. It is important to understand the basic biology of *C. jejuni* pathogenesis, which may lead to novel strategies for circumventing the use of this bacterium as a biowarfare agent. However, the pathogenic mechanisms used by *C. jejuni* to colonize and cause disease in the host are poorly understood. This application is a collaborative proposal between investigators at the University of Houston and UCLA to study the only known exotoxin secreted by *C. jejuni*. We will investigate the cellular mechanisms of the *C. jejuni* cytolethal distending toxin (CDT), which causes cell cycle arrest and eventual death of intoxicated mammalian cells, and has been proposed to assist in remodeling the in vivo environment to facilitate colonization of the intestinal tract. In this R21 application, we will begin to explore the hypothesis that CDT discriminates for and binds to a specific plasma membrane receptor on the surface of sensitive cells as an essential early step during cellular intoxication. In this application, we propose experiments for investigating at the molecular level the interactions of CDT with target cells. In Specific Aim 1, University of Houston researchers will characterize the interactions of CDT with sensitive mammalian cell lines. We will determine the specific and non-specific components as well as the affinity of CDT-receptor interactions. In addition, we will establish many how receptors are present per mammalian cell. Moreover, because CDT is a tri-partite toxin comprising three discrete subunits (CdtA, CdtB, and CdtC), we will establish the contribution of each subunit to binding of the toxin. Finally, we will begin to characterize the nature of the CDT receptor. These experiments will be important for establishing the framework for future experiments to identify the molecular basis for CDT receptor discrimination and binding. In Specific Aim 2, we will identify and characterize mutant cell lines that are resistant to CDT. UCLA researchers will use a genetic approach involving two fundamental phases. In phase 1, cell lines will be mutagenized and screened for a loss of sensitivity to CDT. In phase 2, the loss of sensitivity will be characterized, with the goal being the identification of a cell line that is deficient in binding to CDT. These experiments will be crucial for future work to complement the cell-binding defect, which will identify the putative CDT receptor. Results from this research will provide important information about the mechanism of CDT cellular intoxication, and will provide the basis for future work to develop strategies for blocking the action of CDT as an approach for attenuating *C. jejuni* pathogenesis.

**Grant:** 1R21AI059125-01  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** JOHNSON, ERIC A SCD  
**Title:** Development of Genetic Tools for Clostridium botulinum  
**Institution:** UNIVERSITY OF WISCONSIN MADISON MADISON, WI  
**Project Period:** 2004/05/01-2006/04/30

DESCRIPTION (provided by applicant): Despite the importance of Clostridium botulinum as a human and animal pathogen, and its potential role in bioterrorism, the organism remains extremely poorly characterized with regard to cellular processes and their role in pathogenesis. This is almost entirely a consequence of ineffective genetic systems, and in particular integrational tools. This inability to easily generate stable mutants (directed or random) is common to all clostridial species, and severely hinders the ability of the scientific community at large to fully exploit clostridial genome information in hypothesis driven research. The goal of this project is specifically to develop the necessary enabling technology to overcome this impediment. The aims are: (1) to develop integrational vectors to introduce targeted mutations in genes of C. botulinum; (2) to identify effective transposons for random mutagenesis and (3) to assess antisense RNA strategies for modulation and analysis of C. botulinum gene expression. These aims will be accomplished through the complementary knowledge and expertise of a USA and UK laboratory. The developed genetic tools will pave the way for in depth analysis of not only the C. botulinum genome, but other clostridial genomes too. Their future deployment will, for example, lead to the elucidation of physiological factors that control growth and toxin production, pathogenesis, and developmental processes unique to clostridia and related organisms including endospore formation, resistance, germination and outgrowth.

**Grant:** 1R21AI059185-01  
**Program Director:** CHALLBERG, MARK D.  
**Principal Investigator:** VERARDI, PAULO H PHD  
**Title:** SMART Virus Vectors with a Built-in Safety Mechanism  
**Institution:** UNIVERSITY OF CALIFORNIA DAVIS DAVIS, CA  
**Project Period:** 2004/04/01-2006/03/31

**DESCRIPTION** (provided by applicant): Viruses are powerful tools for the development of vaccines and gene therapies. However, the safety of live recombinant vectors is always a concern, as uncontrolled replication or complications are not inconsequential, particularly in immunosuppressed individuals. In an effort to develop safer, yet still effective live viral vectors, we propose to construct fully replicating virus vectors with a safety mechanism designed to be used when complications with the vector occur or therapy needs to be stopped. Our SMART (Safety Mechanism Assisted by the Repressor of Tetracycline) virus vectors will use elements from the tet operon to regulate the expression of a "safety" gene. Vaccinia virus (VV) is an ideal vector system to test this strategy. The tet system has been successfully adapted to VV, allowing expression to be tightly regulated by the antibiotic tetracycline. In addition, we have shown that interferon-gamma (IFN-gamma) acts as a safety gene in vivo when expressed by VV, attenuating the virus by more than million-fold in immunodeficient mice. Our hypothesis is that live SMART VV vectors expressing a safety gene would be significantly safer: treatment of any adverse reactions would be as simple as antibiotic therapy, since it would allow the expression of the safety gene and significantly enhance virus clearance. More importantly, tetracycline treatment would only be needed when complications occur or are suspected, or when treatment should be stopped. Two specific aims will be addressed under this R21 application: (1) to develop SMART VV vectors expressing a safety gene only in the presence of inducer, and (2) to assess the safety and efficacy of the new vectors. First, SMART VV vectors expressing the tetracycline repressor under a constitutive VV promoter and the reporter gene green fluorescent protein (GFP) under an engineered inducible promoter will be generated and the induction of GFP will be examined in an effort to optimize the system. Then, a SMART vector inducibly expressing murine IFN-gamma, as a model safety gene will be developed. Normal and immunodeficient mice will be given the VV vector expressing IFN-gamma inducibly and survival, pock lesion resolution, disease recovery, weight loss, and virus replication will be assessed in the presence and absence of inducer. In addition, immune responses to VV will be assessed to ensure that the efficacy of the new vectors is not compromised by expression tetracycline repressor expression or tetracycline treatment.



**Grant:** 1R21AI059192-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** COBURN, JENIFER PHD  
**Title:** Vascular tropism of *Borrelia burgdorferi*  
**Institution:** NEW ENGLAND MEDICAL CENTER HOSPITALS BOSTON, MA  
**Project Period:** 2004/04/01-2006/03/31

**DESCRIPTION** (provided by applicant): This application for an NIH R21 award under PA-03-080 (Biodefense and Emerging Infectious Disease Research Opportunities) is based on preliminary studies just recently obtained in the laboratory, and that we believe are of great interest as the basis for further exploratory studies. Our laboratory is involved in the identification of virulence determinants of *Borrelia burgdorferi*, the causative agent of Lyme disease. Lyme disease is considered an emerging infection because the geographic distribution and the number of cases continue to expand in North America. The ability of *B. burgdorferi* to disseminate from the site of inoculation, a tick bite, to multiple tissues indicates that interactions with mammalian cells occur at multiple stages of infection. In particular, the hematogenous dissemination of the spirochete to multiple tissues suggests that interactions with the vascular endothelium are critical to the ability of *B. burgdorferi* to cause infection. As described in this proposal, we used a filamentous phage display library of *B. burgdorferi* DNA in vivo to select for phage clones that exhibit tropism to the vasculature in the hearts and joints of mice. These tissues were selected for analysis because they are frequently infected by *B. burgdorferi* in humans and in animal models of Lyme disease. We have identified three known or predicted outer membrane proteins of *B. burgdorferi* as potential vascular tropism determinants: OspC, VlsE, and the as yet uncharacterized product of gene BB0210. We now propose to generate the relevant fragments of these three proteins in recombinant form, and to test the recombinant proteins for specific binding to endothelial cells. In addition, we will investigate the nature of the endothelial cell receptors for these *B. burgdorferi* proteins. The experiments that we are proposing will shed light on the significance of three *B. burgdorferi* proteins that may facilitate interactions of *B. burgdorferi* with the vasculature during the natural course of infection in mammals.

**Grant:** 1R21AI059233-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** CHEN, ZHENG W MD BIOMEDICAL RESEARCH  
**Title:** T cell immunity to B. anthracis  
**Institution:** BETH ISRAEL DEACONESS MEDICAL CENTER BOSTON, MA  
**Project Period:** 2004/09/30-2004/10/01

DESCRIPTION (provided by applicant): B. anthracis has long been considered a potential biological warfare agent. One of the biodefense research priorities is to develop improved anthrax vaccines. Cell-mediated immune responses may play an important role in vaccine-induced protection against anthrax. Vgamma2Vdelta2 T cells exist only in primates and constitute 60-95% of total human gammadeltaT cell population in the blood. We have recently demonstrated that phosphoantigen-specific Vgamma2Vdelta2 T cells can contribute to adaptive immunity to fatal mycobacterial infection. We have also shown that B. anthracis, like mycobacteria, carries a novel gene encoding GcpE protein that mediates production of phosphoantigen recognized by Vgamma2Vdelta2 T cells. B. anthracis infection of monkeys could prime phosphoantigen-specific Vgamma2Vdelta2 T cells, and result in cross-reactive memory-type responses of these gammadeltaT cells after subsequent infection with phosphoantigen-producing mycobacteria. Since the current anthrax vaccine contains only protein antigens of B. anthracis, phosphoantigen may provide an important addition to anthrax vaccine regimens. We hypothesize that Vgamma2Vdelta2 T cells contribute to both innate and adaptive immune protection against B. anthracis infection. Since peptide-specific CD4 and CD8 T cells may play a role in immunity to anthrax, we further hypothesize that a combined vaccine targeting both phosphoantigen-specific Vgamma2Vdelta2 T cells and peptide-specific CD4 and CD8 T cells are more efficient for immunization and vaccine-induced protection against anthrax. In this R21 application, we will I. Determine vaccine-elicited immune responses of Vgamma2Vdelta2 T cells, and CD4 and CD8 T cells in macaques immunized with a combined vaccine comprised of phosphoantigen and protective antigen (PA) of B. anthracis. II. Determine if combined phosphoantigen and PA immunization of Vgamma2Vdelta2 T cells, CD4 and CD8 T cells can confer protective immunity to inhalation anthrax.

**Grant:** 1R21AI059257-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** MARCONI, RICHARD T PHD  
**Title:** Borrelial Factor H Binding Proteins  
**Institution:** VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA  
**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (provided by applicant): Lyme disease is a zoonotic disease that is transmitted to humans through the bite of infected Ixodes ticks. Lyme disease now represents the most common arthropod borne disease in N. America. Three species of Borrelia are known to cause Lyme disease in humans; B. burgdorferi, B. garinii and B. afzelii. Infection with these bacteria is chronic and can persist for several years. At the present time there is no commercially available Lyme disease vaccine. As a result there is a significant and serious void in the available preventive strategies for Lyme disease. We have recently characterized two proteins that offer great promise for vaccine development. These proteins, FHBP25 and FHBP27 (FHBP25/27), play a pivotal role in Lyme disease pathogenesis by promoting immune evasion through the binding of the complement regulatory protein factor H. Factor H bound to the spirochetal cell surface interacts with factor I which can then cleave the critical complement component, C3b. This decreases the efficiency of the alternate complement cascade, which in turn facilitates the establishment of chronic infection. It is our hypothesis that an FBHP25/27 based vaccine would be superior to other potential vaccines in several regards. First vaccination with FHBP25/27 will result in the production of bactericidal Ab. Second, vaccination will elicit the production of antibodies that would block the ability of FHBP25/27 to bind factor H and thereby render the spirochetes highly susceptible to opsonization and phagocytosis. Lastly, this vaccine has the potential to mediate spirochetes killing in both the tick and mammalian environments. The goal of this application is to determine the efficacy of an FHBP25/27 vaccine and determine its correlates of protection.

**Grant:** 1R21AI059327-01  
**Program Director:** TSENG, CHRISTOPHER K.  
**Principal Investigator:** VANNIEUWENHZE, MICHAEL S PHD  
**Title:** Synthesis and Mechanistic Studies of Peptide Antibiotics  
**Institution:** UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA  
**Project Period:** 2004/04/01-2004/11/30

DESCRIPTION (provided by applicant): Antimicrobial agents that inhibit bacterial cell wall biosynthesis have dominated treatment regimens for the management of bacterial infections for over fifty years. Glycopeptide and beta-lactam antibiotics derive their antibacterial activity through inhibition of key steps in the cell wall biosynthesis cascade. Bacterial resistance to these antibiotics has now reached an alarming level and has underscored the urgent need for new chemotherapeutic agents to augment the cell wall active pharmacopoeia. The objective of this proposal is the chemical synthesis of three very interesting peptide antibiotics (plusbacin A3, katanosin B, and mersacidin) that show very promising antibacterial activity against vancomycin-resistant and methicillin-resistant Gram-positive pathogens. These agents exert their antibiotic action via inhibition of the late-stage reactions involved in peptidoglycan biosynthesis, believed to be the result of sequestration of lipid intermediates utilized in the biosynthetic reactions. We plan to utilize our expertise in the synthesis of cell wall intermediates in order to gain structural information on the antibiotic-lipid intermediate complexes. We will also measure binding affinity of the target compounds, and their derivatives, for various lipid intermediates and attempt to correlate affinity with antibacterial activity and enzyme inhibition. Information gained from these studies will provide valuable insights regarding the function of these agents and may provide a template for de novo design of future-generation antibacterials.

**Grant:** 1R21AI059362-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** HOFT, DANIEL F MD  
**Title:** Vaccine Induced Mucosal Protection Against TB Infection  
**Institution:** ST. LOUIS UNIVERSITY ST LOUIS, MO  
**Project Period:** 2004/04/01-2006/03/31

**DESCRIPTION** (provided by applicant): It is possible that a mucosal vaccine approach that could induce potent mucosal immunity at the point of initial infection in the lower respiratory mucosa could prevent TB infection. By preventing initial TB infection, latent TB infection with the risk for reactivation TB disease would be prevented. There have been no systematic studies of this vaccine concept in any animal model or in humans despite its potential for a huge impact on the overall prevalence of TB infection and disease. In this R21 application, we propose to use modern mucosal vaccination approaches to directly determine whether potent mucosal immune responses can protect against TB infection. Our project will test 2 major hypotheses: 1) Intranasal priming with recombinant mycobacterial proteins and boosting with BCG will induce optimal TB-specific Th1, CTL and secretory IgA responses in the lung. We will use a promising new recombinant TB vaccine candidate, Mtb72f, developed by Corixa Corp. in these studies, alone and in combination with BCG and/or different adjuvants. Dose optimization and prime/boosting experiments will be done giving combinations of recombinant protein, DNA vaccines and BCG intranasally. CpG + cholera toxin will be given as intranasal adjuvants for recombinant protein vaccinations. DNA vaccines will be given intranasally formulated in liposomal preparations. 1 month after the final booster vaccination, lung lymphocytes, spleen cells and bronchoalveolar lavage fluid will be harvested and studied for antigen specific IFN $\gamma$ , CTL and sIgA/IgG responses. 2) Optimal Regional TB-specific Th1, CTL and secretory IgA responses in the lung can provide resistance against initial pulmonary TB infection. The systemic and mucosal prime/boosting strategies found to induce optimal Mtb72f- and BCG-specific immune responses in the experiments outlined above will be studied for the ability to protect against aerosol challenges with mycobacteria. Initially, we will perform aerosol challenges with recombinant BCG expressing a reporter gene (T. cruzi cruzipain vs GFP). In addition to CFU counts, more highly sensitive assays will be used to detect infection in the lungs post-challenge. Real-time PCR assays will be done to detect the presence of the T. cruzi cruzipain gene in lung and peripheral tissues post-challenge. Infection by GFP expressing BCG will be detected by fluorescent evaluation of tissue sections. Prime/boosting strategies found to prevent BCG infection after aerosol challenges will be studied for the ability to prevent infection with virulent M. tuberculosis by aerosol challenge.

**Grant:** 1R21AI059363-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** RICKERT, ROBERT C BS  
**Title:** C3d-PA: efficacy & cellular basis as an anthrax vaccine  
**Institution:** UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA  
**Project Period:** 2004/04/01-2005/03/31

DESCRIPTION (provided by applicant): Numerous factors contribute to the overall effectiveness of a given vaccine; these include specificity, magnitude and tempo of the response, efficiency of inducing memory, and avoidance of reactogenic effects. All of these concerns also apply to the design of adjuvants as a necessary component of virtually all vaccine approaches. This proposal focuses on the application of C3d as a natural molecular adjuvant with increased potency in promoting antigen-specific humoral responses in the absence of co-factors that promote non-specific immunity. Here we will investigate the suitability of C3d conjugation to the protective antigen (PA) of anthrax toxin as a candidate subunit vaccine approach to combat exposure to *B. anthracis* spores or purified exotoxins. C3d-PA fusion proteins and conjugates will be constructed, expressed and tested in mouse in vitro bioassay and in vivo challenge models. Extrapolation of these studies to the activation of PA-specific primary human B-cells will be examined by in vitro rechallenge of circulating memory B cells present in the peripheral blood of military personnel vaccinated during Gulf War I. Together, these studies should reveal the practical effectiveness and mode of action of C3dbound immunogens for potential use in vaccine approaches that require a robust and sustained antibody response. Pending completion of the proposed work outlined in this R21 application, a series of experiments involving anthrax challenge models would seem appropriate.

**Grant:** 1R21AI059436-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** STEWART, GEORGE C PHD  
**Title:** Function of the Bacillus anthracis Spore Carbohydrate  
**Institution:** KANSAS STATE UNIVERSITY MANHATTAN, KS  
**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (provided by applicant): Because of the highly fatal nature of pulmonary anthrax, the ease of production and storage of the spores of *B. anthracis*, and their survival in the environment after an attack, this bacterium has become the primary agent in biowarfare and bioterrorism. The window of opportunity for effective antibiotic treatment of patients with pulmonary anthrax is so small that vaccination is the current best defense against the disease. The spore of *B. anthracis* is the actual infectious agent and the form of the organism that is involved with the first interactions with the host macrophages. Recent studies have found that spore antigens are an important component to immunity against anthrax. We have identified a unique carbohydrate component of the spore. It is not known what roles carbohydrates play in the biology of the spore, from any species of bacteria. In this project, we will create mutants, which deleted for *B. anthracis* spore glycoprotein genes. The effect of these gene knockouts on spore carbohydrate content will be determined by mass spectrometry. The mutant cells will be evaluated for defects in spore formation and the spores from these mutants characterized for their resistance properties and germination efficiency in vitro. The role of the carbohydrate and protein components of the exosporium glycoprotein in conferring immunity to infection will be assessed. Preliminary experiments will be carried out to determine if loss of the BclA glycoprotein of *B. anthracis* affects the virulence properties of this anthrax agent. These studies will provide the information to evaluate whether the spore carbohydrate would be useful as an immunogen in a vaccine against anthrax. The results will provide supporting data for a future proposal to more specifically correlate spore surface structures with virulence and as vaccine targets.

**Grant:** 1R21AI059483-01  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** LEWIS, KIM A PHD  
**Title:** NOVEL METHODS FOR DISCOVERY OF ANTI-MICROBIALS  
**Institution:** NORTHEASTERN UNIVERSITY BOSTON, MA  
**Project Period:** 2004/03/15-2006/02/28

DESCRIPTION (provided by applicant): Our long-term goal is to discover broad-spectrum antibiotics acting against potential biowarfare agents. The goal of this exploratory project is to develop a comprehensive approach to bypass the existing obstacles to drug discovery, which will include delivering active compounds into the pathogen, identifying new classes of antimicrobials, and efficient evaluation of toxicity/efficacy. Previous research in the Lewis laboratory showed that plants synthesize inhibitors of multi-drug resistant efflux pumps that can facilitate delivery of antimicrobials into microbial pathogens. Independently, the Ausubel laboratory developed a pathogenesis model that involves the killing of the nematode worm *Caenorhabditis elegans* by human microbial pathogens. The nematode can therefore be used as an animal model for primary screening of antimicrobials. This proposal describes experiments designed to merge the complementary technologies developed in these two laboratories to produce a novel approach to antimicrobial drug discovery. The Specific Aims are: 1. High throughput whole-animal screen for novel antimicrobials will be developed using *C. elegans* infected with a variety of NIAID group A and B agents. These will include diarrheagenic *E. coli*, *S. enterica*, and a model gram-positive pathogen *E. faecalis*. We will also establish whether *C. elegans* is infected with *B. anthracis* and *F. tularensis*. The rationale of the antimicrobial assay is to monitor curing of worms infected with human pathogens, by test compounds. A liquid assay using GFP-labeled *C. elegans* will be developed into a high-throughput automated assay. 2. Screening for MDR inhibitors and new antimicrobials will be performed in vitro, and in vivo with infected *C. elegans*. A commercial synthetic compound library, and the NCI collection of extracts will be screened. Comparison of the in vitro and in vivo screens will identify possible compounds that only act in vivo (prodrugs, compounds targeting virulence or other components necessary for in vivo survival, and stimulators of innate immunity). Preliminary results show that the NCI library has hits for both direct and MDR inhibitory activity against all pathogens tested. We will focus on obtaining novel antimicrobials and MDR inhibitors acting against gram-negative pathogens. A combination of such an MDR inhibitor with an antimicrobial compound will produce a broad-spectrum antibiotic. We plan to screen 30,000 compounds/extracts in this pilot study. 3. We will purify and identify antimicrobial compounds. Active extracts will be used to isolate a pure substance. A sufficiently pure compound will be used to determine MIC with the pathogen panel, and those with high potency will be studied further. Determination of chemical structure will be performed by a combination of MS and NMR methods.



**Grant:** 1R21AI059489-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** LEWIS, KIM A PHD  
**Title:** BIODEFENSE THERAPEUTICS FROM UNCULTURED MICROORGANISMS  
**Institution:** NORTHEASTERN UNIVERSITY BOSTON, MA  
**Project Period:** 2004/03/15-2006/02/28

DESCRIPTION (provided by applicant): Our long-term goal is to exploit "unculturable" soil microorganisms for production of broad-spectrum antibiotics against potential biowarfare agents. Soil microorganisms have served as the main source of antibiotics, but only 1% grow in vitro, and have been over mined. In this exploratory project, we will develop approaches to culture the bulk of this previously inaccessible biodiversity, based on a method we developed for growing uncultured marine organisms (Kaeberlein, T., Lewis, K., and Epstein, S.S. (2002) Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. Science 296:1127-1129). We also aim to achieve proof-of-principle for obtaining antibiotics from uncultured soil microorganisms. The specific aims are: 1. Growing uncultured microorganisms. Methods for growing soil microorganisms in situ will be optimized. This will form the basis for then developing in vitro culturing methods, based on our findings with marine organisms. The two approaches we will use are "domestication", sequential subculture in a diffusion chamber and subsequent adaptation to growth in vitro; and co-culture with symbiotic organisms+ 2. Screening for antimicrobial activity against BW agents. Extracts from individual microorganisms will be obtained and tested for growth inhibition activity with an avirulent *Bacillus anthracis*\* and LVG strain of *Francisella tularensis*. Hits will be verified against virulent strains of *B. anthracis* and *F. tularensis* (NIAID category A). 3. Purification and identification of antimicrobial compounds. Early-stage dereplication will indicate extracts containing antimicrobial compounds of chemical novelty, and these will be used to isolate a pure substance. A sufficiently pure compound will be used to determine MIC with the pathogen strains, and those with high potency will be studied further. Determination of chemical structure will be performed by a combination of MS and NMR methods.

**Grant:** 1R21AI059503-01  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** HUGHES, KELLY T PHD  
**Title:** Flagellar Phase Variation in Salmonella  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (provided by applicant): Salmonella pathogens infect over a billion people each year worldwide resulting in 3 million deaths annually from septicemia, mostly in HIV-infected patients and 700,000 from typhoid fever (W.H.O. estimates). Salmonella enterica alternatively expresses one of two antigenically distinct flagellin proteins, FliC or FljB. The flagellar filament proteins are major targets of the host immune system, but the role of flagellar phase variation in Salmonella pathogenesis is unclear. The Hin recombinase catalyzes a site-specific DNA inversion event in the *S. enterica* chromosome that controls the alternative expression of flagellin antigens. Upon exposure to HeLa cells, the Hin recombinase is secreted into the extracellular medium and also translocated into the HeLa cells where it is localized into the cell nucleus. Secretion of Hin requires the flagellar Type III secretion system, whereas translocation requires both the flagellar and SPI 1 Type III secretion systems. Also, proper regulation of flagellar genes is essential for Salmonella pathogenesis. The research proposed here will examine the effect of Hin secretion/translocation and Hin-mediated flagellar phase variation on Salmonella pathogenesis, the mechanism of Hin secretion and translocation by the flagellar and SPI 1 Type III secretion systems, the effect of Hin secretion on the regulation of flagellar phase variation, and the effect of the different flagellins on invasion, liver and spleen colonization. In this proposal we will also address the host response to Hin translocation. A particular focus of this grant will be to characterize the role of the Hin recombinase in *S. enterica* pathogenesis and to elucidate the mechanism of Hin secretion and translocation.

**Grant:** 1R21AI059520-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** METODIEV, METODI V PHD  
**Title:** Yeast based model of anthrax lethal factor toxicity  
**Institution:** UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL  
**Project Period:** 2004/09/30-2005/01/01

DESCRIPTION (provided by applicant): The lethal factor of *Bacillus anthracis* is a Zn-endoproteinase that targets the mitogen activated protein kinase kinase (MEK) family of signaling proteins. The toxin recognizes particular members of this family and cleaves a motif that is required for the interaction of MEKs with their downstream effectors--mitogen-activated protein kinases (MAPKs). As a consequence, multiple MAPK pathways are inhibited, and this impairs the viability of affected cells. The goal of this project is to develop an in vivo screening system that allows for the rapid identification of potent and highly specific therapeutics. Rather than screening for compounds that affect particular aspects of lethal factor function in vitro (e.g., its proteolytic activity of the toxin or substrate affinity), we will select for molecules that protect whole cells from lethal factor toxicity using specially engineered strains of the budding yeast *Saccharomyces cerevisiae*. The yeast strains will be designed to conditionally express anthrax lethal factor and to require the function of a human MEK for survival. When expression of the lethal factor is turned on, the MEK will be inactivated and the cells will die. By selecting for the viability of yeast cells, we can screen hundreds of thousands of compounds and identify potent anti-toxins in a matter of weeks. An important advantage of this approach is that it requires maintenance of MEK function. Agents that interfere with MEK signaling will not pass through the screen. Another attractive feature of this system is that in comparison to mammalian cell-based screens, yeast allow for a much faster response to new mutant strains of the pathogen due to its relatively rapid rate of growth. In addition to providing proof of concept, we will optimize the yeast-based system for high-throughput screening of peptide and cDNA libraries, as well as combinatorial libraries of membrane-permeable small molecules.

**Grant:** 1R21AI059541-01  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** JONES, BRADLEY D PHD  
**Title:** Salmonella Invasion Gene Regulation  
**Institution:** UNIVERSITY OF IOWA IOWA CITY, IA  
**Project Period:** 2004/09/30-2005/09/29

DESCRIPTION (provided by applicant): Pathogenic Salmonella strains are an important cause of infectious disease throughout the world. The bacteria possess an invasion-associated type III secretion system, encoded on Salmonella pathogenicity island I, that functions primarily to translocate effector proteins into the cytosol of host cells. These secreted effector proteins have cellular activities that induce uptake of the bacteria into intestinal cells. In addition, the effector proteins have activities that induce fluid secretion and inflammation within the small intestine that are hallmarks of enterocolitis. The expression of the type III secretion system and the associated effector proteins is dependent upon the HilA transcriptional activator, which is a member of the OmpR-ToxR family of transcriptional activators. Overexpression of the hilA gene results in a hyperinvasive phenotype while *S. typhimurium* hilA mutants are up to 500-fold less invasive than wild type strains for tissue culture cells, are significantly impaired in their ability to invade M cells of murine Peyer's patches and have oral LDs<sub>50</sub> values for mice approximately 60-fold higher than the parent strain. The expression of hilA (and the type III secretion system that hilA activates) is regulated by a number of environmental signals (osmolarity, pH, oxygen, growth state) and genetic elements (hilC, hilD, sirA/barA, fliZ, phoBR, fadD, envZ, phoPQ, fis, aroE, pag and bile). Our studies have focused on identifying and characterizing regulators of hilA expression. Recently, we found the fimZ response regulator activates a hile-lacZY reporter. Subsequent work demonstrated that both the phoPQ two component regulator system and the phoBR two component system use the hile/fimZ regulatory pathway to repress hilA expression. As a result, a variety of experiments are proposed to characterize those interactions in detail. In addition, we propose to identify genes regulated by the newly important FimZ response regulator, as well as genes that use FimZ to process signals from the environment. Finally, a previously reported pag repressor gene has been shown to have the ability to regulate the invasion transcriptional activator hilA within tissue culture cells. Experiments are proposed to characterize this repressor in detail as well. To determine the mechanisms by which these regulators affect hilA expression and its associated virulence phenotypes the following specific aims are proposed: 1) Identification and characterization of the signaling pathways that translate environmental signals into alterations in invasion gene expression. 2) Characterization of the FimZ regulon. 3) Identification and characterization of the pag gene and its role in intracellular hilA expression. The overall goal of this proposal is to understand the environmental signals and regulatory mechanisms that control expression of the SPI-1 hilA activator, and the virulence mechanisms that hilA controls including invasion and gastroenteritis. An understanding of this regulatory system will lead to a better understanding of the complex interactions between this pathogen and the host and may lead to the identification of new therapeutic targets to control salmonellosis.

**Grant:** 1R21AI059549-01

**Program Director:** SCHAEFER, MICHAEL R.

**Principal Investigator:** CANNON, JANNE G PHD  
MICROBIOLOGY:BACTERIOLOG  
Y

**Title:** Antigenic Variation in *Francisella tularensis*

**Institution:** UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC  
HILL

**Project Period:** 2004/09/30-2006/08/31

DESCRIPTION (provided by applicant): The intracellular pathogen *Francisella tularensis* (FT) is one of the most virulent bacterial pathogens known, and is capable of infecting humans and many animals. FT could potentially be a dangerous bioweapon; understanding its pathogenesis and developing an effective vaccine for tularemia have therefore become increasingly urgent goals. Nonetheless, remarkably little is known about the pathogenesis of tularemia. One strategy that bacterial pathogens use to enhance their survival during infection is phase or antigenic variation of surface components, which can allow evasion of host defenses. FT shows antigenic variation of lipopolysaccharide (LPS), switching between colony variants with different LPS species: one LPS fails to stimulate nitric oxide (NO) production by rat macrophages, so that the intracellular bacteria survive and grow; LPS of the other variant triggers NO production and efficient killing of the bacteria. The two LPS structures have not been characterized, and nothing is known of the regulation of LPS variation or its role in FT pathogenesis. The long-term goal of this project is to determine how antigenic variation of LPS, and possibly of other components, contributes to the pathogenesis of tularemia and to the ability of FT to evade innate and acquired host defenses. Aim 1 is to initiate studies on LPS antigenic variation by characterizing the structures of the LPS species, identifying the relevant genetic loci, determining the molecular mechanism of antigenic variation, and assessing the effect of LPS variation on the interaction of FT with human cells. It is likely that LPS is not the only antigenically variable component of FT. The FT genome contains a number of genes with the repetitive DNA sequences that are characteristic of phase variable genes in other organisms. Aim 2 is to determine if expression of those genes is indeed subject to phase variation. These studies will provide an initial assessment of the potentially important contribution that antigenic variation makes to the pathogenesis of tularemia.

**Grant:** 1R21AI059557-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** NORTH, ROBERT J PHD  
PATHOLOGY:EXPERIMENTAL  
**Title:** M. bovis as a potentially more virulent MDR pathogen  
**Institution:** TRUDEAU INSTITUTE, INC. SARANAC LAKE, NY  
**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (PROVIDED BY APPLICANT): Virulence may be defined as the ability of a microorganism to survive host defenses and cause disease. There is no reason to believe that multi-drug resistant (MDR) strains of Mycobacterium tuberculosis are more virulent than drug sensitive (DS) strains. There is evidence, on the other hand, that M. bovis is much more virulent than M. tuberculosis as a species. Therefore, if M. bovis were engineered to be MDR, it could pose a more serious threat to public health than MDRM. tuberculosis. The proposed research will use the superior virulence for mice of the Ravenel strain of M. bovis over M. tuberculosis H37Rv to determine whether superior virulence, as manifest by ability to induce faster development of lung pathology and cause earlier death, is associated with the ability to induce a higher level of expression of Th1 immunity in the lung. This will be investigated by measuring levels of expression of genes for Th1 cytokines, proinflammatory cytokines and chemokines in the lungs. Elispot and flow cytometry will be used to enumerate total numbers of IFN- $\gamma$  producing, pathogen-specific CD4 and CD8 T cells in the lungs. The possibility that superior virulence of M. bovis is also associated with higher levels of bacterial gene expression will be investigated, keeping in mind that M. bovis Ravenel and M. tuberculosis cause the same level of stationary lung infection. Real-time RT-PCR will be used to measure levels of pathogen gene expression in terms of mRNA copy number per lung and per CFU. Genes within the RD1 region of the M. tuberculosis and M. bovis chromosome, including *esat6* and *cfp-10*, will receive attention. The virulence of a number of M. bovis and M. tuberculosis strains will be compared.

**Grant:** 1R21AI059563-01  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** SCHOBORG, ROBERT V PHD  
**Title:** INDUCTION OF CHLAMYDIAL PERSISTENCE BY HSV-2  
**Institution:** EAST TENNESSEE STATE UNIVERSITY JOHNSON CITY, TN  
**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (provided by applicant): Chlamydia trachomatis and Herpes Simplex Virus type 2 (HSV-2) are two very common sexually transmitted disease (STD) agents. In the US, 4 million new cases of C. trachomatis and 500,000 new cases of HSV-2 are reported annually. Epidemiological and clinical case studies indicate that double infection with HSV-2 and C. trachomatis occurs in the human population. However, the possibility that co-infection with HSV-2 and C. trachomatis might alter pathogenesis or transmission of one or both of these organisms in vivo has not been rigorously examined. The long-term goal of our laboratory is to determine whether super or double infection with C. trachomatis/HSV-2 changes transmission or disease. As a first step toward this goal, we have established a cell culture model of HSV-2/C. trachomatis co-infection. Our preliminary data demonstrate that HSV-2 super-infection profoundly effects the chlamydial developmental cycle. Chlamydial reticulate bodies (RB) in co-infected cells are swollen and diffuse, resembling persistent chlamydiae. Elementary bodies (EB) are not observed. Co-infected cells also contain numerous membrane blebs; similar structures carry a chlamydial immunodestructive antigen, lipopolysaccharide (LPS), to the surface of persistently infected cells. These data suggest a mechanism by which co-infection could increase chlamydial disease severity by releasing pro-inflammatory molecules and "hyper-activating" the inflammatory response. The immediate goal of this proposal is to test the following hypothesis: productive HSV-2 replication within C. trachomatis infected cervical epithelial cells interferes with chlamydial development and alters release of immunomodulatory molecules from co-infected cells. This hypothesis will be tested in the first 2 related, but independent, Specific Aims. Aim 1 will determine whether productive HSV-2 replication is required for induction of C. trachomatis human serovar E and murine biovar MoPn (C. muridarum) persistent morphology in co-infected cells. Aim 2 will determine whether HSV-2/C. trachomatis co-infected cells produce or release altered quantities of immunomodulatory molecules. Aim 3 will ascertain whether less-pathogenic, attenuated HSV-2 mutants can induce persistence in the human C. trachomatis serovar E as well as the murine biovar MoPn. The studies in Aim 3 will facilitate development of a murine co-infection model system. Completion of this project will narrow down the possible mechanisms by which HSV interferes with the chlamydial developmental cycle, identify immunomodulatory substances released from co-infected calls and set the stage for development of an in vivo routine co-infection model. Additionally, novel mechanisms for induction of chlamydial persistence may be identified, thus increasing our understanding of chlamydial/host cell interactions.

**Grant:** 1R21AI059628-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** BRY, LYNN BA  
**Title:** Protective role of cross-reactive anti-enteric antibody  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (provided by applicant): We hypothesize that the indigenous flora primes adaptive immune responses that can be recruited during primary infection with pathogens that share common or similar cell surface antigens with commensals. We use *Citrobacter rodentium*, a mucosal pathogen of mice, to study the nature and recruitment of these responses. Prior studies by our group have identified that an early T cell-dependent serum antibody response is critical for surviving the acute phase of infection while pre-existing secretory IgA appears to impact early events in colonization, which influence the subsequent severity of infection. This proposal outlines a series of experiments to define antigens from normal flora that elicit immunoglobulin responses against *Citrobacter rodentium*, an attaching and effacing pathogen similar to the EPEC and other class B bioterrorism agents. We will determine the role of serum and mucosal antibody against these shared antigens with the following aims: (1) identify antigens shared between *C. rodentium* and commensal species to which mice naive to infection generate an antibody response, (2) develop monoclonal mucosal (IgA) and serum (IgG or IgM) antibodies against these antigens and assess the ability of administered immunoglobulin to impact colonization and the development of symptomatic infection (3) ascertain protection conferred in wild-type and immunodeficient mice by systemic vaccination strategies using cross-reactive antigens. The results from these experiments will provide a molecular basis for understanding the immunoprotective functions of the indigenous flora, and can assist in the development of better vaccines and therapeutics to provide broad protection against many enteric pathogens of human and veterinary importance.



**Grant:** 1R21AI059639-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** SCHLESINGER, LARRY S MD  
**Title:** TB and Innate Immune Regulation of Lung Macrophages  
**Institution:** OHIO STATE UNIVERSITY COLUMBUS, OH  
**Project Period:** 2004/04/01-2005/03/31

**DESCRIPTION** (provided by applicant): Tuberculosis continues to be an enormous world health problem. Delineation of the specific interactions between the adapted intracellular lung pathogen *M. tuberculosis* (M.tb) and host alveolar macrophages (AMs) in the innate immune response will be important for the development of novel treatment strategies and vaccines. The macrophage mannose receptor (MR) mediates phagocytosis of M.tb. It is highly expressed on AMs and a marker of a unique differentiation state (alternative activation) that includes increased phagocytosis and decreased oxidative responses. AMs are bathed in surfactant protein A (SP-A) and surfactant protein D (SP-D), important lung innate immune molecules. We have determined that SP-A enhances M.tb phagocytosis by upregulating MR function and downregulating macrophage oxidative responses. AMs originate from circulating monocytes that immigrate into the pulmonary microenvironment. We find that SP-A added during monocyte differentiation in vitro leads to a unique phenotype, including enhanced MR and CD14 expression and oxidative responses. In contrast to SP-A, the lesser abundant SP-D agglutinates M.tb and reduces M.tb phagocytosis by macrophages by blocking a microbial ligand for the MR. Our central hypothesis is that M.tb is an efficient respiratory pathogen because it is particularly adapted for survival in the AM whose function is regulated by surfactant components. SP-A, through its action on phagocytes, may enhance the susceptibility of these cells for M.tb entry and growth. Our AIMs are to: 1) To determine the mechanism (s) underlying SP-A-induced up-regulation of MR activity (A) and inhibition of respiratory responses (B) in macrophages. 2) To determine the impact of SP-A, SP-D, and surfactant lipids on the intracellular survival of M.tb in human macrophages. 3) To determine the effects of SP-A on monocyte differentiation to macrophages and the response of these cells to M.tb. Studies will examine those phenotypic and functional attributes characteristic of AM, focusing on regulation of MR activity, the respiratory response, and intracellular survival of M.tb. 4) To determine the role of two key macrophage biochemical mediators in SP-A-induced changes in MR activity and oxidative responses: Protein Kinase C (PKC) and Phosphoinositide-3-kinase (PI3K). We will use microscopy techniques and biochemical assays to assess SP-A's effects on MR trafficking, oxidative responses and SP-A signaling in human macrophages. We will use an in vitro assay of monocyte differentiation to characterize SP-A's effects. Our overall goal will be to better understand the role of surfactant components as regulators of macrophage biology and their impact on M.tb pathogenesis.

**Grant:** 1R21AI059642-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** HILLIARD, GEORGE M PHD  
**Title:** Protein Expression in Strains of *Francisella tularensis*  
**Institution:** UNIVERSITY OF TENNESSEE HEALTH SCI CTR MEMPHIS, TN  
**Project Period:** 2004/09/30-2006/08/31

DESCRIPTION (provided by applicant): *Francisella tularensis* (*F. tularensis*) is a category A bioterrorism agent and is the etiological agent of a multisyndromic disease known as tularemia. Antibiotics are available to treat this disease, but initial diagnosis is difficult. This may lead to recognition delay in the event of a bioterrorist attack with a resultant increase in morbidity. The goal of this proposal is to identify proteins involved in the pathogenic growth cycle of two subspecies of *F. tularensis* (Type A Schu 4 and Type B live vaccine strain (LVS)). Proteomics is a powerful method to identify modulated proteins. Significant proteome coverage can be achieved in organisms with small, sequenced genomes. This proposal outlines plans to exploit recent genomic and proteomic technical advancements by categorizing protein expression in *F. tularensis*; especially establishing the protein expression patterns of two strain types, each with distinctive virulence ranges. The result of this project will be the identification, relative quantitation, and cell localization of the majority of protein products produced by *F. tularensis* in standard broth conditions and after infecting human macrophages. Changes in protein expression patterns will help identify gene products important for *F. tularensis* pathogenesis. This list of virulence candidates will be shared with the research community for subsequent exploration of the pathology of this organism for the purpose of reducing its lethality as an instrument of bioterrorism.

**Grant:** 1R21AI059678-01  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** JUNG, MITCHELL C PHD  
**Title:** Role of PARP-1 in Salmonella Pathogenesis  
**Institution:** GEORGETOWN UNIVERSITY WASHINGTON, DC  
**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (provided by applicant): Salmonella, a potential bioterrorism agent (NIAID Category B Priority Pathogens), can cause life threatening systemic infections. Our objective is to understand the role of poly(ADP-ribose) polymerase-1(PARP-1) in Salmonella pathogenesis. Recently, PARP-1-deficient (PARP-1<sup>-/-</sup>) mice and tissue have been shown to be resistant to inflammatory stimuli. Our preliminary studies indicate that PARP-1<sup>-/-</sup> mice are resistant to Salmonella typhimurium induced septic shock, suggesting that PARP-1 plays an essential role in Salmonella pathogenesis. Additionally, PARP-1 is known to mediate inflammatory-type necrotic cell death and regulate various transcription factors required for the inflammation associated gene expression. These events may be crucial for the outcome of Salmonella infection. Innate immune responses of macrophages are the first-line of defense against infection and therefore a critical parameter for determining the outcome of diseases. However, the role of PARP-1 on innate immune responses of S. typhimurium infected macrophages is currently unknown. Our hypotheses are (a) PARP-1 activity is induced during Salmonella infection and in turn mediates caspase-1-dependent macrophage cell death, and (b) PARP-1 regulates S. typhimurium LPS-induced specific transcription factors and associated inflammatory gene expression in macrophages. Our Aims are to (1) determine the role of PARP-1 in S. typhimurium infected macrophages and on S. typhimurium-induced caspase-1-dependent macrophage cell death, and (2) identify specific transcription factors and proinflammatory genes regulated by PARP-1 in S. typhimurium LPS-activated macrophages. R21 Mechanism: Our project is at an early stage of development, and our preliminary data are limited. The R21 mechanism will allow us to gather critical information regarding the role of PARP-1 on innate immune responses of S. typhimurium infected macrophages. These preliminary studies will be critical for a future R01 to determine the precise role of PARP-1 on Salmonella pathogenesis and to develop methods for the prevention and treatment of infection.

**Grant:** 1R21AI059689-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** FROTHINGHAM, RICHARD MD  
**Title:** Alternative endpoints for plague challenge models  
**Institution:** DUKE UNIVERSITY DURHAM, NC  
**Project Period:** 2004/09/30-2006/08/31

DESCRIPTION (provided by applicant): *Yersinia pestis* causes human and animal plague. Plague vaccine research has increasing importance since *Y. pestis* is a potential bioterrorism agent. Published *Y. pestis* vaccine studies use mortality as the primary endpoint. Mortality endpoints have limited statistical limited power. Since mice are highly susceptible to plague endotoxin and exotoxin, murine mortality may be a poor predictor for human vaccine efficacy. Lastly, lethal challenge models lead to pain and suffering in experimental animals. We will evaluate alternative endpoints to be used in place of mortality in plague challenge models. The first two Aims include the adoption of standard subcutaneous and aerosol models of *Y. pestis* infection and daily measurement of multiple endpoints. Routine endpoints will include mortality, survival time, CFU in multiple sites, and histopathology. Novel endpoints (for plague models) will include comprehensive cytokine profiles and the characterization of host cells associated in vivo with *Yersinia* bacteria. We hope to learn more about plague pathophysiology, and identify better endpoints for measurement of vaccine efficacy. In the third Aim, we will validate selected endpoints by varying components of the challenge models: *Y. pestis* dose, *Y. pestis* challenge strain virulence, active or passive immunization, and nonspecific immune stimulation. Ideal endpoints will have one or more of the following characteristics: (1) consistent within groups, (2) large dynamic range, (3) identifiable early in the course of infection, (4) correlated with input variables (dose, virulence, active or passive immunization, nonspecific immune stimulation) and (5) correlated with mortality. We will evaluate specific hypotheses about the cytokine profiles (early interferon-gamma and tumor necrosis factor-alpha) and responding cell populations (early infiltration of neutrophils, macrophages, and CD69+ cells) associated with protection. We will use funding from this exploratory R21 application to complete the first two Aims and to begin the experiments in Aim 3. We will use the data generated to refine our hypotheses and to pursue further funding to complete Aim 3.

**Grant:** 1R21AI059698-01  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** WALDOR, MATTHEW K MD  
**Title:** Role of Hfq in *Vibrio cholerae* virulence  
**Institution:** TUFTS UNIVERSITY BOSTON BOSTON, MA  
**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (provided by applicant): *Vibrio cholerae* causes the severe diarrheal disease cholera. We found that Hfq, an RNA-binding protein, is essential for *Vibrio cholerae* virulence. Deletion of *hfq* abolished *V. cholerae* colonization of the suckling mouse intestine, but had a minimal effect on growth in vitro and did not influence expression of known colonization factors. Thus, Hfq appears to control previously undescribed pathways essential for cholera pathogenesis. In *E. coli*, Hfq binds to numerous small untranslated RNAs (sRNAs), modulates their activities, and thereby controls expression of a wide variety of genes. Hfq also binds to some mRNAs in *E. coli* and alters gene expression directly. Although Hfq in *V. cholerae* probably acts by similar mechanisms, the distinct phenotypes of *hfq* *V. cholerae* and *E. coli* suggest that the proteins bind different sets of RNAs and control distinct regulons. No RNAs bound by *V. cholerae* Hfq and no *V. cholerae* sRNAs have been characterized to date. The goals of this R21 application are to identify pathways controlled by Hfq in *V. cholerae*, particularly Hfq-regulated genes that contribute to *V. cholerae* virulence, and to characterize the mechanisms controlling their expression. Experiments in Aim I - to define the Hfq regulon - will generate the first knowledge of Hfq-regulated effectors in *V. cholerae*. Experiments in Aim II - cloning of sRNAs and mRNAs that interact with Hfq - will utilize a new, unbiased approach for cloning interacting RNAs. Experiments in both Aims I and II will explore which of Hfq's interaction partners and downstream effectors contribute to *V. cholerae* virulence and thus illuminate currently unknown mediators of pathogenesis. Experiments in Aim III - to match sRNAs to the genes they regulate and characterize processes of Hfq dependent gene regulation - will create the foundation for detailed analyses of the mechanisms by which Hfq controls gene expression in *V. cholerae*. These studies will also facilitate disruption of Hfq-mediated pathways. As Hfq contributes to the virulence of several other Class B Priority Pathogens in addition to *V. cholerae*, Hfq or its downstream effectors may prove to be valuable targets for new antimicrobial agents.

**Grant:** 1R21AI059705-01  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** COFFIELD, JULIE A PHD  
**Title:** Identification of Botulinum Toxin Membrane Targets  
**Institution:** UNIVERSITY OF GEORGIA ATHENS, GA  
**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (provided by applicant): Botulinum toxin, the most poisonous substance known, is one of the top six 'Category A' biological agents. This agent is extremely potent, easy to produce and transport, and has high potential for morbidity and mortality, making it a major threat as a bio-weapon. Currently, there are no readily effective treatment measures for botulism, and death usually results from respiratory muscle paralysis. The diverse health consequences of botulinum toxin combined with the increased threat of bioterrorism underscore the critical need for exploratory research to identify molecular targets that may serve as potential sites for inhibition of toxin action at the clinically relevant target site, the mammalian neuromuscular junction (NMJ). The objective of this exploratory R21 application is to define the membrane target proteins that potentially mediate the selective uptake of botulinum toxin serotypes A and B by nerve terminals of the mammalian NMJ. To achieve this objective co-immunoprecipitation methodology will be combined with proteomic technology to identify proteins, which bind selectively to the toxins. A two-pronged approach will be followed that explores toxin-binding proteins in two different but complementary target tissue preparations, the mouse diaphragm and mouse spinal cord cells. For each target system, two separate sample preparations will be probed. These include 1) a synaptic protein-enriched crude membrane fraction developed in the PI's laboratory and 2) an early endosome-enriched fraction. Once potential membrane receptors for botulinum toxin serotypes A and B are characterized, it will be possible to develop and test toxin antagonists in future studies. If successful, similar protocols could then be applied to the identification of the membrane receptors for the remaining toxin serotypes. Collectively, the results of these studies will significantly impact clinical medicine by defining cellular targets at the NMJ that may serve as templates for the development of effective pharmacologic countermeasures to botulinum toxin exposure and safer toxin-like therapeutic agents.

**Grant:** 1R21AI059798-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** NEPOM, GERALD T  
**Title:** MHC tetramers for epitopes of B anthracis PA  
**Institution:** BENAROYA RESEARCH INST AT VIRGINIA SEATTLE, WA  
MASON  
**Project Period:** 2004/05/01-2006/04/30

DESCRIPTION (provided by applicant): A commitment has been made in the USA to vaccinate large numbers of persons against anthrax. However, limitations of the current anthrax vaccine are widely acknowledged, and efforts are underway to improve the vaccine formulation. We will create biological markers and predictors of the response to anthrax vaccination, with the long-term clinical goal of designing safer and more effective vaccines. To achieve this objective, we will develop novel human class II MHC tetramers which identify antigen-specific CD4+ T cells specific for bacillus anthrax Protective Antigen (BAPA). Quantitation and phenotyping of the tetramer-positive T cells following anthrax vaccination will be evaluated as an indicator of cellular immunity. The Specific Aims are: Aim 1. To identify immunodominant epitopes of bacillus anthrax Protective Antigen (BAPA) based on HLA class II binding and recognized by CD4 T cells which are capable of eliciting robust immunity. Aim 2. To use soluble HLA-BAPA tetramers to quantify the CD4 T cell response to anthrax vaccination. Tetramers will be produced and evaluated which encompass most of the prevalent human class II haplotypes, based on selection of peptides corresponding to T cell recognition of a restricted set of BAPA epitopes. MHC-peptide binding assays, tetramer production and testing methods, and epitope scanning technologies for this project are all validated, and preliminary data using transgenic murine surrogates for the T cell response to BAPA demonstrate successful detection of the responding antigen-specific population following vaccination.

**Grant:** 1R21AI060031-01  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** KULL, F JON AB  
**Title:** Structural Analysis of Bacterial Virulence Regulators  
**Institution:** DARTMOUTH COLLEGE HANOVER, NH  
**Project Period:** 2004/09/30-2005/08/31

DESCRIPTION (provided by applicant): *Vibrio cholerae* causes the frequently fatal epidemic diarrheal disease cholera. The expression of its two primary virulence factors, toxin-coregulated pilus and cholera toxin, occurs via a transcriptional cascade involving several activator proteins and serves as a paradigm for the regulation of bacterial virulence. AphA and AphB initiate the expression of the cascade by an as yet not understood synergistic interaction at the tcpPH promoter. AphA is a member of a new and uncharacterized regulator family and AphB is a LysR-type activator, one of the largest transcriptional regulatory families. Once expressed, cooperation between the homologous transmembrane regulators TcpP/TcpH and ToxR/ToxS activates the toxT promoter. ToxT, an AraC-type regulator, then directly activates the promoters of the primary virulence factors. Transcriptional activation at these various promoters occurs only in response to certain environmental stimuli. Such regulation is widespread among bacterial pathogens and allows productive infections to be mounted only in the appropriate biological niches. The long term goals of the work in this proposal are to understand the molecular basis for this regulation by environmental stimuli, so as to facilitate the development of better strategies to prevent and cure bacterial diseases. Achieving these goals requires an understanding of how the activators themselves function to initiate gene expression and, ultimately, how they are influenced by particular environmental stimuli. Through a collaborative effort of laboratories with expertise in structural biology, virulence gene regulation and pathogenesis, this proposal aims to explore the structure/function relationships of the three cytoplasmic virulence gene regulator proteins in *V. cholerae*, AphA, AphB and ToxT, at their cognate promoters. Specifically, we propose to obtain high resolution structures of (1) AphA; (2) AphB; and (3) ToxT in the absence and presence of their binding sites. In combination with ongoing mutational studies, the proposed work will significantly increase our understanding of how these proteins activate virulence gene expression, will serve as models for these regulatory protein family members in other bacterial pathogens, and will advance efforts to identify molecules that may function as novel therapeutics.



**Grant:** 1R21AI060433-01  
**Program Director:** NEAR, KAREN A.  
**Principal Investigator:** GOLDFELD, ANNE E MD  
**Title:** Clade-specific regulation of HIV-1 by M tuberculosis  
**Institution:** CBR INSTITUTE FOR BIOMEDICAL RESEARCH BOSTON, MA  
**Project Period:** 2004/03/01-2005/02/28

DESCRIPTION (provided by applicant): Tuberculosis (TB) infection is the leading cause of death in the setting of the Acquired Immune Deficiency Syndrome (AIDS). In the human immunodeficiency virus-1 (HIV-1)/TB co-infected host, the pathogenesis and outcome of each infection is greatly influenced by the other. Of the at least 11 subtypes or clades of the major (M) group of HIV-1 strains responsible for the current pandemic, the original clade or subtype isolated, B, has been the most studied. The C and E subtypes, however, are currently the most prevalent globally, accounting for approximately 50% and 30% of all new infections, respectively, and are the most prevalent in those areas of the world also most heavily burdened with TB, Africa and Asia. Notably, there are molecular differences between the B, C, and E subtypes, including distinct long terminal repeat (LTR) and transactivator of transcription (Tat) protein sequences, which are crucial for HIV-1 gene expression, replication, transmission, and disease progression, and it has been suggested that these differences may account for the disproportionate spread of subtypes C and E. Different clinical isolates of the causative agent of TB, *Mycobacterium tuberculosis*, (MTb), also have different phenotypes with respect to their ability to infect, cause disease and elicit cytokine responses including different levels of the cytokine tumor necrosis factor (TNF), which is involved in both the containment of TB disease in the latently infected host and in stimulating HIV-1 replication. Thus, characterization of the regulation of distinct HIV-1 subtypes and their interaction with MTb and differential host production of TNF is critical to the understanding of the pathogenesis of co-infection and to understanding the spread of the C and E HIV-1 subtypes. Our hypothesis, to be tested in Aims 1 and 2, is that LTR-mediated HIV-1 transcription is regulated by MTb in a subtype-specific manner via the recruitment of distinct sets of cellular activators and the remodeling of the LTR chromatin environment in the distinct subtypes. In Aim 3, we will test the hypothesis that subtype specific Tat proteins and diverse MTb clinical strains modulate TNF levels and have distinct effects on the transcription of the HIV-1 B, C and E LTRs. We anticipate that this proposal will result in contributionsto our understanding of the basic mechanisms involved in subtype specific HIV-1 replication by MTb and provide insight into TAT and MTb strain variation upon both HIV-1 and TNF regulation. We also expect that these studies may shed light on why the C clade of HIV-1 is expanding so rapidly in countries with a high TB burden. The long range goal of these studies is to identify subtype-specific transcriptional targets to inhibit HIV-1 replication in the setting of MTb infection and/or T cell activation secondary to MTb specific host immune responses.

**Grant:** 1R21AI060725-01  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** OJCIUS, DAVID M AB  
**Title:** Abortion and innate immunity in chlamydial infection  
**Institution:** UNIVERSITY OF CALIFORNIA, MERCED MERCED, CA  
**Project Period:** 2004/09/01-2006/08/31

DESCRIPTION (provided by applicant: Chlamydia species provoke serious infections of humans and animals worldwide, despite extensive work to better characterize the biology of infection. Chlamydiae also cause abortions in many domestic animals, and infection by Chlamydia and other microbial pathogens is thought to lead to a large but undetermined fraction of miscarriages and preterm births in humans. Little is known about how Chlamydia infection leads to sterility, and in general, the mechanisms leading to abortion have not been characterized during infection by any microbial pathogen. Recent studies on abortion in model systems in the absence of infection have shown that fetuses are rejected mainly by the innate immune system, via inflammation and complement activation. Secretion of Th2 cytokines at the fetal-maternal interface also favors successful pregnancy, while the pro-inflammatory Th1 cytokines, which are required for elimination of chlamydial infection, are harmful to the fetus. Finally, stimulation of CD1d-dependent natural killer T (NKT) cells by injection of a CD1d ligand induces abortion through a mechanism requiring NKT cell-mediated cytotoxicity and pro-inflammatory cytokines. Our preliminary data show that Chlamydia infection induces abortion in mice, even though the fetus itself is not infected. Chlamydia infection leads to complement activation (C3 deposition) in the uterus of non-pregnant mice, and in the labyrinthine zone of the placenta of pregnant mice. Expression of the NKT cell receptor is weak in the uterus or the placenta of uninfected mice, but increases greatly in the placenta of infected mice. The overall hypothesis to be tested is that the innate immune system, via complement and/or NKT cell activation, could lead to abortion in Chlamydia-infected mice. Our first two goals will be to characterize Chlamydia infection in C3- and CD1d-deficient mice, which have not been studied yet in the absence of pregnancy. We will then characterize the effects of complement activation and NKT cell activation on the outcome of pregnancy during Chlamydia infection.

**Grant:** 1R21AI060726-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** POPHAM, DAVID L PHD  
**Title:** SPORE PEPTIDOGLYCAN DEGRADATION IN BACILLUS ANTHRACIS  
**Institution:** VIRGINIA POLYTECHNIC INST AND ST UNIV BLACKSBURG, VA  
**Project Period:** 2004/04/01-2006/03/31

**DESCRIPTION** (provided by applicant): The infectious agent in anthrax is the dormant *B. anthracis* spore, and establishment of infection requires germination and outgrowth of the spore. Degradation of the spore cortex peptidoglycan wall during germination is necessary to allow rehydration and resumption of metabolism in the spore core. Cortex lytic enzymes are present in an inactive form in the spore and are activated only in the presence of specific germinant molecules. Goals of the proposed studies are identification of the important *B. anthracis* cortex lytic enzymes, characterization of their activities, and understanding of the mechanisms for their activation. Structures of the peptidoglycan found in *B. anthracis* dormant and germinating spores will be determined in order to identify cortex lytic activities operating during germination. Genes predicted to encode cortex lytic enzymes, based upon sequence similarities, will be disrupted and changes in germination lytic activities in the mutant strains will be characterized. Cortex lytic enzymes will be extracted and purified from germinating *B. anthracis* spores. The enzymatic activities and identities of these proteins will be determined. A greater understanding of the process of spore cortex lysis will suggest possible methods for combating anthrax infection on two fronts. Identification of drugs or treatments that block cortex lysis will allow treatment of individuals exposed to spores to prevent establishment of infection. Identification of chemicals or treatments that activate cortex lysis, resulting in germination and rendering the spores susceptible to standard bactericidal agents, will allow easier decontamination of spore-contaminated sites and prevention of human exposure.

**Grant:** 1R21AI060762-01  
**Program Director:** PETERS, N KENT  
**Principal Investigator:** GUSAROV, IVAN PHD  
**Title:** NO Metabolism: a New Target for Antimicrobial Therapy  
**Institution:** NEW YORK UNIVERSITY SCHOOL OF MEDICINE NEW YORK, NY  
**Project Period:** 2004/06/01-2006/05/31

**DESCRIPTION** (provided by applicant: In their natural environment pathogenic bacteria are persistently exposed to reactive oxygen and nitrogen species generated by a host. Forced to withstand this stress, pathogens have developed a sophisticated defense system. Despite extensive research, the role of nitric oxide (NO) in host-pathogen interaction remains unclear. Various reports have registered both deleterious and cytoprotective effects of NO. Our preliminary data indicate that Gram-positive bacteria employ exogenous NO to immediately activate their oxidative stress defense system by a novel mechanism. The objectives of this proposal are to clarify the role of host generated NO for pathogen survival and design the methods to enhance the bactericidal properties of NO. Our proposed experiments are based on sufficient preliminary data and expected to yield, within provided time frame, significant results which will be applicable for treating a broad spectrum of pathogens. Specific aims are: 1. Understand the mechanism of NO-mediated bacterial cell protection and its role in host-pathogen interaction. Our in vitro and in vivo studies demonstrate that a key enzyme of bacterial oxidative stress defense system, the catalase, is strongly and directly activated by physiological amounts of NO. Using *Staphylococcus aureus* as a model pathogen, we will determine a mechanism of NO-dependent catalase activation and its role in bacteria evasion of immune response. It is further proposed using small molecules to compromise the bacterial catalase activation thus improving the effectiveness of the innate immune response to a variety of Gram-positive pathogens. 2. Design specific NO-inducible antibiotics. Since pathogenic bacteria are exposed to large quantities of host-derived NO and also generate their own NO, the design of specific small molecules that become cytotoxic upon reaction with NO provides a conceptually new way to combat infection. Our preliminary studies demonstrate that the hydrophobic derivatives of 5-aminonaphtalenesulfonamides (ANSA) can efficiently suppress various enzymatic activities upon interaction with NO and posses strong bactericidal effect. It is further proposed to develop selective antimicrobial compounds based on ANSA.

**Grant:** 1R21AI060770-01  
**Program Director:** KORPELA, JUKKA K.  
**Principal Investigator:** BRITIGAN, BRADLEY E  
**Title:** Use of Gallium to Prevent Pseudomonas Biofilm Formation  
**Institution:** UNIVERSITY OF CINCINNATI CINCINNATI, OH  
**Project Period:** 2004/09/01-2006/08/31

DESCRIPTION (provided by applicant): Biofilm formation is important in the pathogenesis of acute and chronic infections of the lung by *Pseudomonas aeruginosa*, decreasing the effectiveness of host defenses and bacterial susceptibility to killing by antibiotics. Recent evidence shows that limiting iron (Fe) availability prevents *P. aeruginosa* biofilm formation and enhances its susceptibility to tobramycin. Gallium (Ga), a group IIIA transition metal disrupts the Fe metabolism of many cell types. We have shown that Ga inhibits the growth and Fe acquisition by *Mycobacterium tuberculosis*. Based on these and new data, we hypothesize that Ga can disrupt *P. aeruginosa* Fe acquisition/metabolism, thereby disrupting biofilm formation and enhancing susceptibility to conventional antibiotics. It may also limit the growth of planktonic bacteria that would be in dynamic equilibrium with the biofilm at sites of *P. aeruginosa* infection. Thus, Ga could become a potent therapy against *P. aeruginosa* biofilms. To test these hypotheses, two specific aims will be pursued. First, we will confirm and define the mechanism whereby Ga inhibits *P. aeruginosa* biofilm formation and ascertain if Ga enhances the susceptibility of *P. aeruginosa* to conventional antibiotics. This will be done using two in vitro *P. aeruginosa* biofilm models. Second, we will determine the effect of Ga on *P. aeruginosa* Fe acquisition and Fe-dependent gene regulation. We will directly measure Fe and Ga acquisition by *P. aeruginosa*, as well as characterize the interaction of Ga with its siderophores. DNA microarray technology will be used to study the impact of Ga on *P. aeruginosa* gene regulation. The long-term goal of this work is to develop a means to utilize Ga for the prevention and/or treatment of *P. aeruginosa* biofilms and the infections that result. We would envision the systemic and/or aerosolized pulmonary delivery of Ga to *P. aeruginosa*-infected patients and/or impregnating endotracheal tubes with Ga to prevent biofilm formation in ventilated patients.

**Grant:** 1R21AI060862-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** BARKER, LUCIA P BOTH  
**Title:** Molecular characterization of *M. marinum* biofilms  
**Institution:** UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN  
**Project Period:** 2004/06/01-2006/05/31

**DESCRIPTION** (provided by applicant): The mycobacterial species, *Mycobacterium marinum*, is a marine pathogen that infects poikilothermic animals such as fish and frogs and will also infect humans. *M. marinum* can form biofilms both in the laboratory and in the environment, but little is known about the molecular factors responsible for *M. marinum* biofilm formation. In this application, we describe the use of a flow-cell system for the study of *M. marinum* biofilm formation in situ. We will quantify biofilm formation and observe deposition of organisms upon a plastic matrix using both standard and fluorescence microscopy. We will further harvest *M. marinum* biofilms grown in flow cells for use in a differential fluorescence induction (DFI) system. In this way, we will be able to isolate promoters specifically induced during biofilm formation. In a complimentary approach to the DFI studies, we will isolate proteins from harvested *M. marinum* biofilms and the planktonic (free-floating) counterparts within the same culture and subject these proteins to 2-dimensional gel electrophoresis. This will allow the identification of mycobacterial proteins specific to biofilm formation. Promoters and proteins isolated will be compared to known *M. marinum* sequences as well as sequences available in other mycobacterial databases. We will, using these methods, be able to identify specific genes required for mycobacterial biofilm formation. In addition, we will be able to visualize the location of specific gene products within the biofilm using the same reporter assay system and confocal microscopy on the flow-cell-generated biofilm. These studies will lead to an understanding of the mechanisms by which mycobacteria form biofilms in nature and on artificial surfaces, and a more specific understanding of how biofilms can contribute to the pathogenesis of these organisms.

**Grant:** 1R21AI060898-01  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** KHAN, SALEEM A  
PHD  
BIOCHEMISTRY:BIOCHEMISTR  
Y-UNSPEC  
**Title:** Genomics/Proteomics of Enterotoxin B Producing *S. aureus*  
**Institution:** UNIVERSITY OF PITTSBURGH AT PITTSBURGH PITTSBURGH, PA  
**Project Period:** 2004/07/01-2006/06/30

DESCRIPTION (provided by applicant): *Staphylococcus aureus* produces a number of enterotoxins (SEs) which are the causative agents of staphylococcal food poisoning. The genes for SEs are located on plasmids, phages and other potentially movable genetic elements such as pathogenicity islands, further increasing their possible spread to other organisms in the environment. Staphylococcal enterotoxin B (SEB) is an NIAID Category B Priority agent and is generally produced at much higher levels than other SEs by toxigenic *S. aureus* strains. Furthermore, the levels of SEB produced by different *S. aureus* strains are highly variable. Recent studies suggest that SEB may regulate the synthesis of other extracellular proteins including virulence factors, thereby affecting staphylococcal virulence. The goal of this Exploratory/Developmental proposal is to study the gene expression and proteomic profiles of various SEB-producing *S. aureus* strains and to further test the hypothesis that expression of SEB by *S. aureus* strains may affect the production of intracellular and extracellular proteins, including other toxins and virulence factors. This will be accomplished by a comparative genomic analysis of different SEB-producing strains utilizing *S. aureus* microarrays provided to us by NIAID through TIGR/PFGRC. We will also study the cellular gene expression profiles in these strains using microarrays. The effect of SEB on global gene expression in isogenic *S. aureus* strains will also be studied utilizing the microarray technology. We will also study the proteomes of SEB-producing *S. aureus* strains as well as pairs of isogenic SEB-positive/SEB-negative strains by two-dimensional Difference Gel Electrophoresis using fluorescence dyes. Differences in the protein content and levels of particular proteins in these strains will be identified by mass spectrometry. Successful completion of these studies will provide a solid basis for future analysis of the molecular mechanisms involved in the regulation of SEB production, and its effect on staphylococcal gene expression and pathogenesis.

**Grant:** 1R21AI060941-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** DAVIS, GEORGE E MD MEDICINE  
**Title:** Function of CMG-2, an Anthrax Toxin Receptor  
**Institution:** TEXAS A&M UNIVERSITY HEALTH SCIENCE COLLEGE STATION, TX  
CTR  
**Project Period:** 2004/06/01-2006/05/31

DESCRIPTION (provided by applicant): Recent studies have discovered two cell surface-expressed anthrax toxin receptors (ATRs), originally named TEM8 and CMG-2. The latter receptor, CMG-2, was first discovered in my laboratory in a screen for genes regulating the process of capillary tube morphogenesis in three-dimensional collagen matrices. Intriguingly, both receptors were identified as upregulated genes during the process of new blood vessel formation (i.e. angiogenesis). In addition, recent work in collaboration with my laboratory has revealed that CMG-2 is mutated in two familial human diseases, juvenile hyaline fibromatosis and infantile systemic hyalinosis. CMG-2 shows characteristics of a type I transmembrane receptor with a von Willebrand factor (vWF) type A domain in its extracellular domain and potential cytoskeletal interacting domains in its cytoplasmic tail. The vWF domain of CMG-2 was shown by us to bind the extracellular matrix ligands laminin and collagen type IV and recently was shown to bind protective antigen (PA83), the known ATR binding component of the tripartite anthrax toxin. We hypothesize that CMG-2 associates with co-receptors such as integrins to mediate its normal function in extracellular matrix homeostasis as well as its function as an ATR. Clearly, molecular studies involving structure-function analyses of the novel alternatively spliced receptor, CMG-2, are required to elucidate the mechanisms underlying its ability to serve as an ATR. Also, it is critical to identify CMG-2 binding proteins, such as cell surface co-receptors and cytosolic binding partners, which regulate its function. Such studies are critical toward the development of novel therapeutic agents that interfere with the ability of anthrax toxin to exert its toxic effects. We present preliminary data showing the novel involvement of  $\alpha_5\beta_1$  integrins in endothelial cell adhesion and spreading on protective antigen-coated wells (mimicking the presentation of extracellular matrix ligands). These data strongly suggest that integrin co-receptors may play a role in the binding, processing and internalization of protective antigen. The specific aims of this application are: Specific Aim #1. To perform structure-function analyses with CMG-2 splice variants, mutants, and domains (i.e. extracellular versus cytoplasmic) to determine which domains are involved in protective antigen binding, proteolytic processing and internalization. Specific Aim #2. To identify CMG-2 co-receptors, such as integrins, which are involved in its ability to regulate protective antigen binding, proteolytic processing and internalization.



**Grant:** 1R21AI060968-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** BOYER, JULIE BS  
**Title:** Genetic Passive Immunotherapy for Shiga toxin  
**Institution:** WEILL MEDICAL COLLEGE OF CORNELL UNIV NEW YORK, NY  
**Project Period:** 2004/09/30-2006/08/31

DESCRIPTION (provided by applicant): Passive immunotherapy is used to treat intoxication by a variety of biological toxins. Exposure to toxigenic bacteria or purified toxin preparations in a bioterrorism event would necessitate administration of therapeutic antibodies in a mass response setting. The standard practice of intravenous infusion of antibodies to exposed individuals as well as the expense associated with large preparations of purified antibodies would not be practical in this situation. Therefore, a strategy is presented for rapid protection against a bacterial toxin with adenovirus (Ad)-based vectors that express neutralizing single chain antibodies. Shigatoxin was selected for these proof-of-principle experiments due to the availability of specific reagents and the classification of shigatoxin and *E. coli* O157, a shigatoxin-producing bacterial strain, as potential biowarfare threats. The general principle is that Ad-based gene transfer vectors expressing neutralizing single chain antibodies can elicit rapid passive immunity against shigatoxin by a convenient route of administration and that this technology can be applied to other potential toxin threats. The proposal is based on our experience in the development of Ad-based anti-bacterial vaccines and the ability of Ad vectors to deliver single chain antibodies against specific pathogens. The 3 specific aims outline studies to achieve these goals by developing Ad vectors that express a shigatoxin-specific single chain antibody and optimizing the binding and neutralization characteristics of the single chain antibodies through genetic manipulation and evaluation in an in vivo challenge system. Aim 1: To evaluate the hypothesis that shigatoxin-specific single chain antibodies expressed by Ad vectors can neutralize shigatoxin. Aim 2: To refine the technology of Ad-expressed antishigatoxin single chain antibodies, modifications known to increase the stability, avidity, and molecular weight of anti-shigatoxin single chain antibodies to enhance their protective efficacy will be examined. Aim 3: Evaluate the ability of Ad-delivered anti-shigatoxin antibodies to protect experimental animals from challenge with shigatoxin and toxigenic bacteria.

**Grant:** 1R21AI060976-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** BIER, ETHAN  
**Title:** Genetic Analysis of Bacterial Toxins in Drosophila  
**Institution:** UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA  
**Project Period:** 2004/06/01-2006/05/31

Drosophila is the invertebrate genetic model system most closely related to humans and is emerging as a powerful tool for analyzing the function of human disease genes. Among genes highly conserved between Drosophila and humans are those encoding targets for bacterial toxins such as those causing anthrax, cholera, and tetanus. There is significant concern that several bacterial toxins, including anthrax LF and cholera toxin CTX-A1, could also be used in biological weapons. These toxins enter host cells and can cause irreversible damage or death, even after the pathogenic bacteria have been eliminated. An important contribution to fighting the pathogenesis of such toxins would be to develop agents inhibiting their activity within the host cell. We have recently developed a genetic method in Drosophila called "Novel Overexpression Allele (NOVA)" screening, that permits the efficient isolation of dominant alleles in a gene of interest. An important element of this exploratory/developmental R21 grant is to initiate a new line of research in my laboratory in which we use the NOVA method to generate dominant negative (DN) alleles of bacterial toxin genes expressed in Drosophila. These DN alleles, which interfere with wild-type toxin function, may be useful in developing reagents to treat patients exposed to bacterial toxins and may serve as tools to further define the mechanism of toxin action. In line with another key objective of the R21 funding mechanism, we hope to obtain the necessary preliminary data for submitting an RO1 grant to continue and extend these studies. This grant has the following specific aims to generate and characterize DN-toxin alleles: Aim 1. Express A-type subunits of bacterial toxin genes in Drosophila 1-1: Subclone bacterial toxin genes into the pUASW2 expression vector 1-2: Generate phenotypes by expressing toxins in specific subsets of Drosophila cells 1-3: Characterize phenotypes resulting from expression of pUASW2-toxin transgenes Aim 2. Screen for dominant negative (DN) alleles of bacterial toxin genes in Drosophila 2-1: Mutagenize flies pUASW2-toxin transgenes and use NOVA to screen for DN alleles 2-2: Characterize molecular lesion in DN-toxin NOVA alleles 2-3: Confirm that DN-toxin alleles interfere with wild-type toxin gene function 2-4: Determine whether DN-toxin alleles function in mammalian cells

**Grant:** 1R21AI061006-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** CANGELOSI, GERARD PHD  
**Title:** Genetic Analysis of Mycobacterial Biofilm Formation  
**Institution:** SEATTLE BIOMEDICAL RESEARCH INSTITUTE SEATTLE, WA  
**Project Period:** 2004/07/01-2006/06/30

DESCRIPTION (provided by applicant): The Mycobacterium avium complex (MAC) is the most significant of the environmental mycobacteria that cause disease in susceptible humans. Most MAC infections are thought to come from stably colonized drinking water supplies. The predominant way of life for bacteria in water is growth in surface-adherent biofilms, and recent studies have shown that MAC is no exception. It is present in vast numbers in biofilm samples taken from water distribution systems, and it appears to be the dominant Mycobacterium species in such environments. Genetic approaches have been used to analyze biofilm formation by the fast-growing model species M. smegmatis; however the genetics of biofilm formation by MAC remains to be explored. Using newly developed genetic and genomic tools, combined with authentic laboratory models of MAC biofilm development, we will conduct the first such investigation. The specific aims of this exploratory project are 1) to identify MAC genes involved in the early stages of biofilm formation by using a novel "transposome" mutagenesis approach, and 2) to identify MAC genes involved in the formation of mature two-species biofilms by using signature-tagged mutagenesis and transposon site hybridization. By establishing methods and conditions for genetic analysis of biofilm formation by MAC, we will begin to shed light on an aspect of mycobacterial biology that is almost entirely uncharacterized. The results will improve our understanding of the microbial ecology of environmental mycobacteria, and they may lead to improved methods for protecting susceptible people from MAC infection.

**Grant:** 1R21AI061020-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** GIRON, JORGE A PHD  
**Title:** Novel Type IV Pili of Enterohemorrhagic E. coli O157:H7  
**Institution:** UNIVERSITY OF ARIZONA TUCSON, AZ  
**Project Period:** 2004/09/30-2005/09/29

DESCRIPTION (provided by applicant): Enterohemorrhagic E. coli (EHEC) O157:H7 is recognized as an important emerging pathogen responsible for producing hemorrhagic colitis and the hemolytic uremic syndrome (HUS) in humans. EHEC O157:H7 strains elaborate a potent Shiga toxin, which has been associated with the pathogenesis of HUS. No pili have yet been reproducibly identified in O157:H7 strains and therefore, it is still an enigma as to whether pili play a role in colonization of the intestine of their natural bovine or accidental human hosts. We have recently identified a novel pilus produced by EHEC strain EDL933 and other O157:H7 strains. These pili, herein called hemorrhagic coli pili 1 (Hcp1), belong to the virulence-associated type IV pili family. Further, purified Hcp1 showed agglutination of rabbit erythrocytes and bound to fibronectin and laminin suggesting an adhesive role in binding to human proteins. An isogenic Hcp1-deficient mutant showed considerably low levels of adherence to cultured cells. The pilin monomer is encoded by the chromosomal prepilin peptidase-dependent *ppdD* gene, which is adjacent to the type IV-like piliation genes *hofBC*. Based on our preliminary data, we hypothesize that Hcp1 is a key virulence factor that contributes to the adhesive properties of EHEC. Overall, the objective of this proposal is to advance knowledge of EHEC pathogenesis by elucidating the mechanism(s) of adherence of EHEC O157:H7 to human epithelial cells in culture. Several multidisciplinary approaches involving molecular biology, cell biology, ultrastructural analysis by high power electron microscopy, and biochemical and antigenic analysis will be carried out to extend our current knowledge on the interaction of EHEC O157:H7 with host target cells. The outcome of this proposal will provide important implications for detection of E. coli O157:H7 in food sources and reservoirs and importantly for prevention and control of EHEC infections in humans. The central focus of this proposal lies in structure-function studies of Hcp produced by EHEC. Thus, the following specific aims are proposed: 1) To define the genes required for Hcp1 biogenesis; 2) To define the role of Hcp1; 3) To analyze expression of *ppdD*.

**Grant:** 1R21AI061026-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** THOMPSON, STUART A BS  
**Title:** Campylobacter jejuni DNA methylation and gene regulation  
**Institution:** MEDICAL COLLEGE OF GEORGIA (MCG) AUGUSTA, GA  
**Project Period:** 2004/09/30-2005/08/31

DESCRIPTION (provided by applicant): Campylobacter jejuni is a Category B Bioterrorism Agent as classified by the NIH. It is the leading cause of bacterial gastroenteritis in the United States, and is responsible for sporadic disease as well as food-borne and water-borne outbreaks. There are at least 2.4 million cases of C. jejuni gastroenteritis in the U.S. annually, with an incidence exceeding that of Salmonella and Shigella combined (1). C. jejuni infection is also the most common antecedent event to development of Guillain-Barr Syndrome, an acute motor paralysis apparently resulting from an autoimmune response directed at C. jejuni surface antigens. Despite the high prevalence of Campylobacter disease and more than 20 years of study, the mechanisms by which C. jejuni causes disease remain obscure. Several C. jejuni virulence factors have been identified, yet their regulatory mechanisms are largely unknown. Recent evidence implicates DNA methylation as an important signal controlling virulence gene expression in Salmonella and other bacteria. In light of this, our studies on the predicted C. jejuni DNA methylase Cj1461 showed that cj1461 was induced at 37C, the internal temperature of humans, consistent with a role in pathogenic growth adaptation. Further experiments revealed that mutation of cj1461 resulted in the dramatically altered expression of ca. 50-60 proteins, including known virulence factors and additional predicted regulatory proteins such as the orphan response regulator Cj0355. Consequently, Cj1461 appears to be involved in a complex regulatory circuit controlling expression of numerous C. jejuni genes; growth temperature is one of the signals. We now propose further study of gene regulation in C. jejuni, focusing on those proteins that are regulated by the predicted DNA methylase Cj1461 and the orphan response regulator Cj0355. We will use a combination of microarray, proteomics, and biochemical approaches to achieve the goals outlined in these three specific aims: Specific Aim 1. Determine the functional properties of Cj1461 and Cj0355 responsible for modulating virulence gene expression. Specific Aim 2. Identify the downstream targets of Cj1461 and Cj0355 to define their combinatorial impact on gene regulation. Specific Aim 3. Elucidate the upstream factors and signals controlling Cj1461 and Cj0355 expression to reveal the entire regulatory circuit.

**Grant:** 1R21AI061051-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** CRAIG, LISA BS  
**Title:** Structure and Assembly of Type IVb Pili  
**Institution:** SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA  
**Project Period:** 2004/06/15-2004/12/31

DESCRIPTION (provided by applicant: Diarrheal diseases kill 2.2 million people annually, mostly children, and are the second largest cause of death worldwide. *Vibrio cholerae*, *Salmonella*, and the enteropathogenic and enterotoxigenic *E. coli* (EPEC and ETEC respectively) cause diarrheal diseases that are devastating to people in developing countries and also pose a serious threat to international travelers and military personnel. In addition these organisms present a health threat here in the United States and are listed as Category B agents of bioterrorism by the Centers for Disease Control. The virulence of these organisms is attributed, in part, to the Type IV pili, which allow the bacteria to form microcolonies and colonize the human intestine. Our x-ray crystallographic results on the pilin subunits that comprise the pili suggest that the two Type IV pilin subclasses, IVa and IVb, are structurally distinct. We generated a molecular model for the Type IVb toxin coregulated pilus (TCP) from *V. cholerae* by integrating crystal structure and packing data with the filament dimensions and helical symmetry of the TCP filaments. This model is generalizable for all Type IV pili whereby the conserved N-terminal  $\alpha$ -helices anchor the structurally variable pilin heads, which contribute surface variation for specificity of pilus function in antigenicity, motility, adhesion and microcolony formation. In this application we intend to structurally characterize Type IVb pili from the enteric pathogens *V. cholerae*, *S. enterica* serovar Typhi, EPEC and ETEC. We plan to solve the crystal structures of several N-terminally truncated Type IVb pilins, and to determine their orientation in the pilus filaments using a newly emerging and powerful method of structural analysis, deuterium exchange mass spectrometry. These data will be integrated to derive molecular models for the Type IVb pili using the TCP model as a template. Knowledge of the structure and assembly of these key virulence factors will provide insight into their colonization functions and a basis for the design of vaccines and therapeutics.

**Grant:** 1R21AI061058-01  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** MAURELLI, ANTHONY T.  
**Title:** Metabolic Modeling of Invasive Bacteria and HeLa Cytosol  
**Institution:** HENRY M. JACKSON FDN FOR THE ADV ROCKVILLE, MD  
MIL/MED  
**Project Period:** 2004/07/15-2006/06/30

DESCRIPTION (provided by applicant): Most of the bacteria in the NIAID Priority Pathogen list from all three Categories invade human cells and for many the only known reproductive stage of infection takes place in membrane-bound vacuoles or directly in the cytosol of these host cells. Almost all antibiotics, and all that are administered orally, act by blocking some metabolic pathway of rapidly growing bacteria rather than by disrupting the bacterial cell. Therefore, the identification of new targets for antibiotic action requires knowledge of the active metabolism of replicating intracellular (IC) bacteria. Predictive computer models of the metabolism of *E. coli* (a close relative to *Shigella flexneri*) have been constructed using Constraint-based Flux Balance Analysis (CFBA). The chemical constituents of rapidly growing *E. coli* cells have been determined, and this information was used in building the CFBA models. However, the equivalent information for eukaryotic cells, which is basically the growth medium for IC bacteria, is not as complete, and will be investigated. Knowledge of the constituents of eukaryotic cell cytosol is required to develop CFBA models to analyze the metabolism of bacteria during their IC replicative stage. *S. flexneri*, a Category B pathogen, will be used as: (Specific Aim 1) a biosensor to determine constituents of the cytosol of human-derived cells and (Specific Aim 2) the model organism for this innovative application of CFBA. Several attributes of *S. flexneri* make it a good choice to probe the eukaryotic cytosol for bacterially accessible compounds: replication in the cytosol (no vacuolar membrane to complicate the analysis), a large repertoire of uptake systems (to assess availability to bacteria of compounds in the cytosol) and genetic tools to examine heterologous uptake systems. CFBA translates a metabolic network (described as stoichiometric bio-chemical reactions) into an optimization problem with constraints on each molecule that the organism can exchange with its environment (in this case eukaryotic cytosol). A general model (to allow adaptation to different bacteria) will be constructed from the Kyoto Encyclopedia of Genes and Genomes (KEGG) metabolic database. The *S. flexneri* CFBA model will be derived from the reference model using published reports of the genome and the IC behavior of *S. flexneri* mutants. Modeling the metabolism of NIAID Priority Pathogens will aid our understanding of a large number of potential bioterror agents and help direct the search for new antibacterial drugs.

**Grant:** 1R21AI061073-01  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** GOLDBERG, MARCIA B MD  
**Title:** Autotransporter periplasmic chaperones in Shigella  
**Institution:** MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA  
**Project Period:** 2004/07/01-2006/06/30

DESCRIPTION (provided by applicant): "Autotransporter" proteins are the largest family of secreted proteins among gram-negative bacteria. A large number of autotransporter proteins, including several that are present among Category B Priority Pathogens, are known to have significant roles in pathogenesis. A distinguishing feature of autotransporters is the ability to mediate the translocation across the outer membrane of an intramolecular passenger (alpha) domain that performs a specific activity in the extracellular space. Our data indicate that at least one autotransporter (Shigella IcsA) is folded in the periplasm and suggest that it may remain folded during outer membrane translocation. Published data indicate that the periplasmic chaperone DegP is required for efficient secretion of IcsA. In this R21 application, we propose to explore whether periplasmic chaperones are generally required during secretion of autotransporters. Our studies will focus on the DegP family of protease chaperones and the five autotransporters of the Category B Priority Pathogen Shigella. Aim 1. Exploration of whether DegP serves as a periplasmic chaperone of Shigella autotransporters generally; Aim 2. Exploration of whether the DegP homologs DegQ and DegS also function as periplasmic chaperones of Shigella autotransporters; and, Aim 3 Determination of whether the conserved C-terminal motif of autotransporters is recognized by DegP, DegQ, and DegS. These exploratory studies are highly likely to result in additional specific testable hypotheses relevant to disease caused by this Category B Priority Pathogen.



**Grant:** 1R21AI061106-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** WEINSTOCK, GEORGE M PHD  
**Title:** Francisella Genomics  
**Institution:** BAYLOR COLLEGE OF MEDICINE HOUSTON, TX  
**Project Period:** 2004/07/15-2005/06/30

DESCRIPTION (provided by applicant): The goal of this project is to determine the DNA sequence of *Francisella tularensis* subspecies *holarctica*, the causative agent of tularemia. *F. tularensis* has been classified as a Category A Select Agent posing a serious military and civilian bioterrorism threat. Two strains of *F. tularensis* are currently being sequenced: a highly virulent type A strain and a type B (*holarctica*) strain used as a live vaccine. This project will determine the sequence of the genome of the parent of the vaccine strain. This strain is expected to retain intact virulence factors that have been altered in the vaccine strain, leading to its attenuation. Thus comparison of this sequence with that of the live vaccine strain as well as the type A strain will identify candidate genes for virulence factors. Moreover, sera from vaccinated individuals should be useful in identifying antigens in future studies, using the genomic sequence of the type B strain. Early results from genomic studies suggest that *F. tularensis* contains a high proportion of unique genes compared to other bacterial genomes. Therefore, knowledge of the complete genome sequences of *F. tularensis* strains is needed for the understanding of the virulence mechanisms.

**Grant:** 1R21AI061155-01  
**Program Director:** KLEIN, DAVID L  
**Principal Investigator:** CHATTOPADHYAY, DEBASISH PHD  
**Title:** Structure of Surface Antigens of *S. Pneumoniae*  
**Institution:** UNIVERSITY OF ALABAMA AT BIRMINGHAM BIRMINGHAM, AL  
**Project Period:** 2004/07/01-2006/06/30

DESCRIPTION (provided by applicant): *Streptococcus pneumoniae* is the main cause of acute otitis media, sinusitis, pneumonia, meningitis and bacteremia. Together, these infections cause well over one million deaths worldwide. Rapid spread of penicillin resistant and multiple antibiotic resistant strains of bacteria worldwide pose serious public health threat. The clinical efficacy of 23-valent polysaccharide vaccine is less than ideal, failing to protect individuals at high risk, such as children and the elderly. Although the conjugated 7-valent vaccine, currently licensed in some countries seems promising, it still needs long term clinical evaluation. Moreover, these vaccines are too expensive for the developing world, where the threats for these infections are highest. Our long-term goal is to design a protein based vaccine against pneumococcal infection. A recombinant protein based vaccine has the advantage of being more effective in children and can be produced at low cost. The goal of this proposal is to elucidate the three dimensional structure of a major surface antigen and virulence factor of *S. pneumoniae*, namely Pneumococcal Surface Protein A, (PspA). PspA is a potential vaccine candidate. It is highly immunogenic. Antibodies elicited against PspA are cross-reactive and protective against different strains of pneumococci. It has been used to immunize humans and human anti-PspA antibodies can protect mice from sepsis. PspA inhibits killing of pneumococci by lactoferrin by binding to it. A detailed knowledge of the three dimensional structure of PspA would help us understand the structure function relationship among its epitopes. This knowledge would be extremely valuable for designing effective vaccine based on PspA. Moreover, structure of PspA with human lactoferrin would allow designing novel strategies to block PspA-lactoferrin interaction. Towards these goals, the specific aims of this proposal are: 1) Determine the crystal structure of PspA. 2) Determine the structure of PspA-lactoferrin complex.

**Grant:** 1R21AI061396-01  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** WOZNIAK, DANIEL J PHD MICROBIOLOGY, OTHER  
**Title:** The matrix of *Pseudomonas aeruginosa* biofilms  
**Institution:** WAKE FOREST UNIVERSITY HEALTH WINSTON-SALEM, NC  
SCIENCES  
**Project Period:** 2004/07/01-2005/06/30

DESCRIPTION (provided by applicant): A critical element of *Pseudomonas aeruginosa* pathogenesis is the ability to form biofilms in the lungs of cystic fibrosis (CF) patients and on many other surfaces. Bacteria within biofilms produce one or more extracellular polymeric substances (EPS) that stabilize the biofilm and act as a scaffold. Alginate has been considered the major polysaccharide of the biofilm EPS matrix. However, recent studies indicate that alginate is not involved in the initiation of biofilm formation by non-mucoid *P. aeruginosa* strains, which are the first to colonize CF patients and are the cause of most acute *P. aeruginosa* infections. In CF patients, mucoid conversion typically occurs months or years after initial colonization. There remain significant gaps in understanding how *P. aeruginosa* survives the harsh, inflammatory-rich environment of the CF lung prior to converting to the alginate-producing phenotype. The central hypothesis to be examined is that *P. aeruginosa* has the capacity to express alternative EPS molecules that are essential for biofilm formation and persistence of *P. aeruginosa* during infection. This application will focus on one alternative polysaccharide, designated Eps1, which plays a critical role in biofilm formation. The overall objective is to determine the role of Eps1 in biofilm development, structure, resistance to antimicrobial agents, and *P. aeruginosa* pathogenesis. Aim 1 will focus on defining the genes within the *eps1* gene cluster that are required for biofilm formation and understanding the role of Eps1 in formation of the biofilm matrix. In the second aim, the regulation of the *eps1* gene cluster will be examined and the hypothesis that the *eps* genes are spatially and temporally controlled during biofilm development will be tested. Finally, experiments in aim 3 will determine if Eps1 contributes to the persistent phenotype of biofilms and is thus important in *P. aeruginosa* virulence. A further understanding of this critical biofilm component will lead to strategies aimed at inhibiting biofilm formation, which is a key aspect of *P. aeruginosa* pathogenesis.

**Grant:** 1R21AI061399-01  
**Program Director:** VAN DE VERG, LILLIAN L.  
**Principal Investigator:** MAHAN, MICHAEL J PHD  
**Title:** Epigenetic Control of Bacterial Virulence  
**Institution:** UNIVERSITY OF CALIFORNIA SANTA BARBARA SANTA BARBARA, CA  
**Project Period:** 2004/07/01-2006/06/30

DESCRIPTION (provided by applicant): Epigenetic control of gene expression is employed in animals and plants to enable genetically identical cells to pursue different fates, and such cell differentiation is central to their development. Epigenetic modification and information transfer may also be employed during the bacterial infection cycle so as to change the expression of virulence functions temporally and spatially within the host. These alterations in gene expression may accompany bacterial dissemination among host sites, and in response to host inflammation, tissue breakdown, and immune reactions. In Aim 1, we propose to test the hypothesis that a methylation-based epigenetic system controls *Salmonella* pathogenesis. Our proposal is based on altering the expression of the bacterial methyltransferase, termed DNA adenine methylase (Dam), which is involved in the formation of DNA methylation patterns and is known to be required for virulence in a number of pathogens. We propose to determine whether transient overexpression of Dam activity in *S. typhimurium* leads to the establishment of a lineage of genotypically wild-type, epigenetically modified cells that exhibit altered DNA methylation patterns and altered virulence states that persist for a number of cell generations. If so, the present state of virulence gene expression in a given bacterial cell may be dependent on its past history (i.e., cellular memory), similar to that observed in cell-differentiation and developmental programs in higher organisms. In Aim 2, we propose to specify the mechanism by which cellular memory control occurs at the level of a single gene using the *S. typhimurium* pef operon as a model system. Plasmid encoded fimbriae (Pef) expressed by *S. typhimurium* mediate adherence to intestinal epithelium and are required for virulence. The pef operon is under methylation-dependent transcriptional regulation similar to the well characterized *E. coli* pap operon, which encodes important virulence determinants in urinary tract infections. We propose to test whether Dam overproduction-mediated defects in pef expression are a direct consequence of altered DNA methylation patterns that map to specific upstream pef Dam-target sites (GATC sequences). We further propose to determine whether transient exposure to dam overexpression leads to persistent changes in Pef synthesis and whether such changes are heritably maintained via altered DNA methylation patterns at upstream Per GATC sites. The finding of a methylation-based memory system in bacteria would provide profound insights into the fundamental molecular mechanism(s) underlying control of virulence gene expression and the resultant changes in pathogen behavior that are critical to infection. Such information is vital toward developing novel antimicrobials and vaccines against biowarfare agents and emerging infectious diseases that currently threaten public health worldwide.

**Grant:** 1R21AI061426-01  
**Program Director:** PETERS, N KENT  
**Principal Investigator:** BOWDEN, MARIA G PHD  
**Title:** Structure and Function of S. Epidermidis Adhesins  
**Institution:** TEXAS A&M UNIVERSITY HEALTH SCIENCE COLLEGE STATION, TX  
CTR  
**Project Period:** 2004/06/01-2006/05/31

DESCRIPTION (provided by applicant): *Staphylococcus epidermidis* is an emerging human pathogen that infects implanted medical devices, specially those present in immunocompromised patients. Despite its importance as a human pathogen, the virulence factors of *S. epidermidis* are not well characterized. Since *S. epidermidis* does not produce many exotoxins, we propose that its cell wall associated adhesions are essential for effective bacterial colonization and pathogenicity. Therefore, adhesins are attractive targets in the development of novel strategies to prevent and treat infections. *S. epidermidis* expresses two cell-wall anchored proteins, SdrG and SdrF, that are predicted to be adhesins and are similar to the *S. aureus* fibrinogen (Fg) binding MSCRAMMs. SdrG binds to the N-terminal residues of the Fg beta chain and is necessary for the attachment of *S. epidermidis* to immobilized Fg. We have solved the crystal structures of the Fg-binding region of SdrG as an apoprotein and in complex with a Fg-derived peptide. Based on these structures, we propose that SdrG changes its conformation upon ligand binding, in a series of events described in the "dock, lock and latch" model. We propose that Gram-positive MSCRAMMs that have a SdrG-like predicted structure may bind to linear, peptide-like ligands with a similar mechanism. We have begun to test the "dock, lock and latch" model. We propose to use several conformation-probing techniques testing SdrG as a model molecule, and subsequently apply the same principles and probe the conformational changes of other SdrG-like MSCRAMMs. In addition to the structural analysis, we will develop a murine catheter-infection model to explore the role of specific Fg-binding MSCRAMMs in *S. epidermidis* infections. Curiously, SdrG only binds with high affinity to human fibrinogen. Since *S. epidermidis* is exclusively a human pathogen, we will test if the SdrG specificity for human fibrinogen contributes to the *S. epidermidis* human tropism. Finally, we will examine the role of another SdrG-like protein, SdrF, as a virulence factor in *S. epidermidis* infections.

**Grant:** 1R21AI061432-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** STRALEY, SUSAN C  
PHD BOTANY  
NEC:BIOPHYSICS  
**Title:** Surface proteins in pneumonic plague  
**Institution:** UNIVERSITY OF KENTUCKY  
LEXINGTON, KY  
**Project Period:** 2004/07/01-2006/06/30

DESCRIPTION (provided by applicant): *Yersinia pestis* is the most highly virulent extracellular pathogen known and poses an ominous threat as an agent of bioterrorism. The bacteria are highly infectious by aerosol and cause pneumonic plague, which can kill in as little as two days after exposure. There is no vaccine available for plague. Vaccines under development for prevention of plague contain two proteins called LcrV and F1. They provide moderate protection against pneumonic plague, but not if the infecting strain lacks the F1 capsular protein. F1 is not required for virulence by the aerogenic route, and its gene is easily deleted, posing the potential of a weaponized F1-lacking strain against which these candidate vaccines do not protect effectively. To find alternative vaccine candidates, we will develop a novel proteomics approach to identify *Y. pestis* surface proteins that are expressed during pneumonic plague. These studies will provide the technical base for a more detailed screen for new plague vaccine candidates. Further, we hope that one or more of the proteins we identify in the proposed pilot studies will prove in the future to be protective against pneumonic plague caused by a non-encapsulated strain against which vaccines currently under development will not protect adequately. Our aims are: 1. Develop a biotinylation-MS approach for identification of surface-exposed proteins on *Y. pestis* recovered from lungs of mice with pneumonic plague. 2. Identify up to 10 surface proteins and evaluate them for conditions that optimize expression.

**Grant:** 1R21AI061454-01  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** BESSEN, DEBRA E PHD  
**Title:** Molecular Evolution of Resistance in Strep. pyogenes  
**Institution:** NEW YORK MEDICAL COLLEGE VALHALLA, NY  
**Project Period:** 2004/06/01-2006/05/31

DESCRIPTION (provided by applicant): Group A streptococci (GAS; *Streptococcus pyogenes*) are highly prevalent bacterial pathogens that infect the throat or skin of humans, causing pharyngitis or impetigo. GAS can trigger autoimmune disease, such as rheumatic fever, or cause life-threatening infections, such as toxic shock syndrome. Macrolide-resistance in GAS developed abruptly in the 1980s and accelerated in the 1990s, exceeding 30% of GAS isolates in some regions. The long-term goal of the proposed study is to understand the molecular mechanisms underlying the emergence, spread, and persistence of macrolide resistance in GAS. The proposed research combines epidemiology, molecular genetics, and basic evolutionary principles in novel ways. Aim 1 will seek to define the molecular characteristics of macrolide-resistant clones of GAS collected from throughout the world. Using nucleotide sequence determination, isolates will be defined for emm-type, resistant genes and housekeeping loci, via multilocus sequence typing. A database will be maintained on the Internet, to facilitate worldwide surveillance. Aim 2 will seek to trace the recent evolutionary history of newly emerged resistant clones. Evolutionary models can be used to address several novel issues: (a) estimate the number of times resistant strains arose, (b) evaluate the significance of immune escape in the spread of resistance genes, (c) uncover bias in the order of acquisition of multiple resistance genes, and (d) identify problematic clones and lineages based on their evolutionary trajectory. By tracking nucleotide polymorphisms arising via recombination, a new molecular archaeological approach will also be developed, to predict which part(s) of the world resistance most likely emerged, and the geographical routes of clonal spread. Future studies can complete the evolutionary framework for all macrolide-resistant clones, extend the questions on genetic dynamics (a-d, above), and assess differences for efflux versus methylase resistance mechanisms. The high global prevalence of GAS, combined with its high rate of genetic recombination, makes it ripe for quickly evolving in unexpected ways. GAS provide a sound model system for understanding the molecular evolution underlying the early stages of the emergence and global spread of antibiotic resistance.

**Grant:** 1R21AI061520-01  
**Program Director:** SCHMITT, CLARE K.  
**Principal Investigator:** NATARO, JAMES P  
**Title:** Dispersin from Enteroaggregative E. coli  
**Institution:** UNIVERSITY OF MARYLAND BALT PROF SCHOOL BALTIMORE, MD  
**Project Period:** 2004/09/30-2005/08/31

DESCRIPTION (provided by applicant): Enteroaggregative E. coli is an emerging enteric pathogen, whose virulence mechanisms are only partially elucidated. We have shown that EAEC adherence to the human intestinal mucosa is mediated by Aggregative Adherence Fimbriae (AAFs), but that the strong autoagglutination phenotype mediated by AAF fimbriae is modulated by a hydrophilic protein coat that is non-covalently attached to the surface of the bacterium. The coat protein is a 10.2 kDa species which we have named dispersin, the product of the aap gene on the EAEC virulence plasmid. We have recently solved the solution structure of dispersin and have shown it to be a tightly folded rod-like structure with two prominent beta-sheets. We have discovered that translocation of dispersin to the bacterial cell surface requires a putative ABC transporter complex, which includes a cognate homolog of the outer membrane channel TolC. We have proposed a molecular model for the AatA channel based on the TolC crystal structure. Given the novelty and importance of dispersin to EAEC pathogenesis, and the emerging importance of TolC family members and ABC transporters, we propose to characterize further both the structure-function aspects of the dispersin protein as well as functional characteristics of its secretion. These studies will provide contributions to understanding of each of these important areas. Work will be organized in three distinct aims. Aim 1: Structure-function analysis of dispersin. Here, we will address requirements for secretion and for function. The NMR structure will be used to build hypotheses and NMR spectroscopy will be used to evaluate the effects of mutagenesis. Aim 2: Structure-function analysis of AatA. In this aim, we will verify the TolC-based model of the AatA translocator of dispersin. We will also perform site-directed mutagenesis of specific regions of AatA suggested to be important and to confer diversity of function compared with TolC itself. Aim 3: Characterization of the inner membrane ABC complex. In aim, we will characterize the inner membrane complex required for dispersin secretion, the atypical ABC transporter represented by AatPBCD. Each protein will be localized and their roles addressed using mutagenesis and biochemical strategies.



**Grant:** 1R21AI061533-01  
**Program Director:** TAYLOR, CHRISTOPHER E.  
**Principal Investigator:** GRANOFF, DAN M. MD  
**Title:** Neisseria meningitidis antigens expressed in infection  
**Institution:** CHILDREN'S HOSPITAL & RES CTR AT OAKLAND, CA  
OAKLAND  
**Project Period:** 2004/06/01-2006/05/31

DESCRIPTION (provided by applicant): *N. meningitidis* is an important cause of meningitis and sepsis. Conventional approaches to develop a vaccine for prevention of disease caused by capsular group B strains, which account for 30-80% of all cases, have been largely unsuccessful, and therefore there is a need for the identification of new vaccine candidates. To date, all candidate antigens have been identified using organisms grown in artificial media or on epithelial or endothelial cell monolayers. Little is known, however, about the gene expression profile of *N. meningitidis* during infection in vivo. Our hypothesis is that *N. meningitidis* grown in the infant rat model of bacteremia or in human whole blood will show specific genes that are up-regulated or activated compared to those expressed in artificial culture. Furthermore, genes that are up-regulated during invasive infection that encode novel proteins that are conserved in *N. meningitidis*, and that are predicted to be surface-accessible, will be promising vaccine candidates. We propose to use quantitative real-time PCR to study the temporal gene expression profile of *N. meningitidis* isolated from the bloodstream of infected infant rats and from the human blood infection model at different time-points, and also to compare the respective expression profiles with those of bacteria grown in broth. We will evaluate the immunogenicity of the recombinant proteins as purified His-tagged or GST-fusion proteins, and as "native" proteins in *E. coli* outer membrane vesicles. Antisera from immunized mice will be analyzed by ELISA, FACS, serum bactericidal activity, and passive protection in the infant rat model. The proposed studies may identify new antigens capable of eliciting broadly protective antibody for prevention of *N. meningitidis* disease, including group B strains for which there is currently no vaccine available. Also, characterization of gene expression in an in vivo model will lead to a better understanding of meningococcal pathogenesis.

**Grant:** 1R21AI061538-01  
**Program Director:** QUACKENBUSH, ROBERT L.  
**Principal Investigator:** VALDIVIA, RAPHAEL H PHD  
**Title:** Functional Genomic Analysis of the Chlamydia Inclusion  
**Institution:** DUKE UNIVERSITY DURHAM, NC  
**Project Period:** 2004/05/20-2006/04/30

DESCRIPTION (provided by applicant): Chlamydia trachomatis is a widely disseminated, obligate intracellular pathogen that causes a range of diseases including trachoma, conjunctivitis and pelvic inflammatory disease. Within infected cells, C. trachomatis efficiently re-routes endocytic and exocytic traffic to create a growth-permissive compartment termed the Inclusion. Because Chlamydiae are not currently amenable to genetic manipulation, little progress has been made in identifying bacterial or host factors required to reprogram vesicular traffic in the host cell. Nonetheless, the increasing availability of Chlamydia genomic sequences provides the opportunity for the design of new experimental approaches to study Chlamydia pathogenesis. We propose a functional genomic approach to identify factors required for the biogenesis and maintenance of the C. trachomatis Inclusion. We propose to perform a three-stage screen of Chlamydia expression libraries in model eukaryotic systems to identify bacterial factors that interfere with endocytic traffic. Because C. trachomatis-mediated disruption of endocytic traffic is limited to the Inclusion, we hypothesize that the function of many Inclusion proteins is restricted to the surface of endosomal membranes. Therefore, a central aspect of our expression system includes anchoring Chlamydia proteins to the surface of endosomes (Endosomal Display). In the first screening stage, we utilize whole cell-based assays in the yeast *Saccharomyces cerevisiae* to rapidly sample all Chlamydia proteins of unknown function for factors that disrupt eukaryotic cellular functions. In the second stage, Chlamydia proteins that display a phenotype in yeast are expressed in Chinese Hamster Ovary (CHO) cells to confirm and/or extend phenotypes observed in yeast. We will raise antibodies to these Chlamydia proteins to determine their subcellular localization during Chlamydia infections. In the last stage of the screen, we will use the antibodies and cell lines generated to determine if these factors are necessary for Chlamydia growth in host cells. A third of the Chlamydia genome encodes proteins whose function cannot be determined from sequence analysis. We expect that the functional approach we propose will lead to the identification of novel classes of virulence factors and represent a major breakthrough in our understanding of Chlamydia pathogenesis.

**Grant:** 1R21AI061555-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** XU, YI PHD  
**Title:** Cell wall protein in Bacillus anthracis pathogenesis  
**Institution:** TEXAS A&M UNIVERSITY HEALTH SCIENCE COLLEGE STATION, TX  
CTR  
**Project Period:** 2004/07/01-2006/06/30

**DESCRIPTION** (provided by applicant): Anthrax is an old disease with re-born relevance to both scientists and public. To fight this old foe, the causative organism, *Bacillus anthracis*, must be understood clearly and fully at the mechanistic molecular level of its pathogenesis. Many Gram-positive pathogenic bacteria possess cell wall anchored proteins (CWAPs) that are critical for virulence and are excellent vaccine candidates. By analogy, the CWAPs of *B. anthracis* are likely to show equal relevance and utility. Analysis of the *B. anthracis* genome revealed nine previously uncharacterized CWAPs. Preliminary studies indicated a recombinant fragment of one of these recognized specific macrophage targets. Macrophages play a central role in the establishment of anthrax. Two additional CWAPs were found to bind collagen, which is a major component of the skin where cutaneous anthrax develops. Together these findings support the hypothesis that CWAPs of *B. anthracis* are significant in its pathogenesis. The two specific aims of this proposal are to determine the roles of CWAPs in the interaction of *B. anthracis* with 1) macrophages and 2) major skin components i.e., collagen and fibroblasts. To achieve these aims, deletion mutants will be generated for each of the nine CWAPs. The mutants will be evaluated for their ability to associate with, be engulfed by and survive within macrophages, as well as their ability to adhere and invade human dermal fibroblasts. The respective mutants will also be used to determine the relevance of each of the two collagen-binding CWAPs in the adherence of *B. anthracis* to collagen. To confirm the function of the CWAPs, the deleted genes will be complemented then expressed in a heterologous host. Their molecular targets in the host cells will subsequently be identified. In the future, the effect of these proteins in *B. anthracis* virulence will be evaluated in an animal model in collaboration with Dr. Theresa Koehler at University of Texas Medical School, and Dr. Rick Lyons at University of New Mexico. The long-term objectives are to elucidate the biological functions of these proteins, their molecular interactions with the host and their potential as vaccine and drug targets. The information will likely improve the understanding of the infection mechanisms of *B. anthracis*, and may provide novel effective ways to combat the scourge of anthrax.

**Grant:** 1R21AI061590-01  
**Program Director:** PETERS, N KENT  
**Principal Investigator:** JEFFERSON, KIMBERLY K BS  
**Title:** Antibiotic resistance of *S. aureus* biofilms  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 2004/06/15-2006/05/31

DESCRIPTION (provided by applicant): *S. aureus* is an important cause of biofilm-related infections such as endocarditis, chronic osteomyelitis, corneal infections involving lens implants, and medical device-related infections. Infections involving staphylococcal biofilms are often very difficult to treat with antibiotics and may be recurrent or require surgical removal of the infected device or tissue. Antibiotic susceptibility tests used in the clinical laboratory measure the resistance of planktonic or free-floating bacteria and do not account for the fact that *S. aureus* biofilms are significantly more resistant to most antibiotics than planktonic bacteria. The characteristics of biofilms that make them refractory to antibiotic therapy are not well understood at this time. The long-term objective of this project is to characterize the properties of staphylococcal biofilms that make them refractory to antimicrobial chemotherapy. The immediate goal of the proposed project is to assess the role in antibiotic resistance of poly-N-acetyl glucosamine (PNAG), which plays a critical role in *S. aureus* biofilm formation. First, the influence of PNAG expression on antibiotic resistance of planktonic bacteria will be characterized. Next, a PNAG-independent biofilm model, based on the cross linking of bacteria via protein A / Fc-receptor interactions, will be implemented so that the role of PNAG in biofilm antibiotic resistance can be separated from its role in biofilm formation. In addition, the effect of PNAG on the penetration of fluorescently labeled antibiotics throughout biofilms will be analyzed by confocal microscopy. Finally, the interaction between PNAG and antibiotics commonly used to treat *S. aureus* infections will be characterized. We hypothesize that PNAG plays an important role in the resistance of *S. aureus* biofilms by acting as a physical barrier against antibiotic penetration and expect that knowledge of the role of PNAG in *S. aureus* biofilm resistance to antibiotics will advance the development of therapies used to combat these infections.

**Grant:** 1R21AI061602-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** CROSA, JORGE H PHD CHEMISTRY:CHEMISTRY-UNSPEC  
**Title:** Iron Uptake and Virulence of Burkholderia pseudomallei  
**Institution:** OREGON HEALTH & SCIENCE UNIVERSITY PORTLAND, OR  
**Project Period:** 2004/07/15-2006/06/30

**DESCRIPTION** (provided by applicant): Melioidosis and glanders are life threatening infectious diseases with similar patho-physiology caused respectively by the select agents *Burkholderia pseudomallei* and *B. mallei*, with dramatic repercussions in the field of bio-defense. Despite major recent advances including the elucidation of the entire genome sequence of *B. pseudomallei* K96243 and the ongoing sequencing of the *B. mallei* genome, little is known about their mechanisms of virulence. Our long-range goal is to elucidate the pathogenesis of these diseases. Although many factors can contribute to bacterial virulence, one important nonspecific mechanism of defense pathogenic bacteria must overcome to establish infection, is the ability of human and animal hosts to withhold iron. Thus, a competitive advantage for microorganisms is the possession of genetic determinants encoding products that allow them to utilize otherwise unavailable iron. The immediate goal of our research is to use a combination of genetic and biochemical approaches to unveil specific mechanisms of iron uptake employed by these pathogens during both the septicemic and the intracellular phases of disease. To accomplish these goals we will perform: 1. Microarray and mutational analysis. We will use microarray chips to examine the differential gene expression of *B. pseudomallei* K96243, from cells grown in vitro under conditions of iron repletion and limitation. We will also examine patterns of gene expression induced in *B. pseudomallei* during infection of macrophages. In conjunction with microarray analysis we will generate knock-out mutants of important iron uptake genes in *B. pseudomallei*, identified from the available genome sequencing project. Selected iron uptake mutants will be assessed for their subsequent ability to invade and survive intracellularly in macrophages and for virulence in animal models. 2. The characterization and structural analysis of siderophores from *B. pseudomallei* and *B. mallei*. We will characterize the structure of siderophores produced by wild type and mutant *B. pseudomallei* (K96243) and *B. mallei* (ATCC 23344). These compounds will be purified by high performance liquid chromatography and their structural details determined by nuclear magnetic resonance and mass spectrometry. Knowledge gained from our investigation will contribute to the development of measures to control the diseases caused by these pathogens.

**Grant:** 1R21AI061606-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** MCDONOUGH, KATHLEEN A. PHD  
**Title:** Differential Gene Expression in *Yersinia pestis*  
**Institution:** WADSWORTH CENTER RENSSELAER, NY  
**Project Period:** 2004/06/15-2006/05/31

DESCRIPTION (provided by applicant): The long-term objective of this proposal is to better understand the molecular basis of *Y. pestis* differentiation from the closely related species *Y. pseudotuberculosis* (Yptb), particularly with respect to its ability to cause disease. This proposal addresses the hypothesis that differential gene expression in response to environmental conditions is a critical determinant that distinguishes the plague bacillus from Yptb in nature. The specific aims are to: Aim 1: compare and contrast, using 2D-gel electrophoresis, patterns of *Y. pestis* and Yptb protein expression in a variety of biologically relevant conditions; Aim 2: identify, using mass spectrometry (MS), a subset of *Y. pestis* proteins that are differentially expressed in response to the environment. This group will include proteins that are expressed specifically by *Y. pestis*, as well as some that are similarly regulated by both *Y. pestis* and Yptb. MS analyses will be done on: a) proteins isolated from 2D gels analyzed in Aim 1; b) differentially-tagged and fractionated whole cell protein lysates; Aim3: perform preliminary characterization of selected genes from Aim 2, using molecular and bioinformatics approaches to evaluate each gene's potential for future study. *Yersinia pestis*, the etiologic agent of plague, is a major biological threat agent and a Class A select agent. Identification of *Y. pestis* proteins that are expressed during biologically relevant conditions will provide new targets that can be exploited for plague detection, treatment, and prevention, as well as insights into the pathogenesis of this critically important pathogen. Characterization of *Y. pestis*-specific genes and their proteins in future studies will also identify potential mechanisms by which *Y. pestis* emerged as a new pathogen distinct from Yptb.

**Grant:** 1R21AI061702-01  
**Program Director:** BAKER, PHILLIP J.  
**Principal Investigator:** LARNER, ANDREW C MD  
**Title:** The Effects of Interferons on Anthrax Toxicity  
**Institution:** CLEVELAND CLINIC LERNER COL/MED-CWRU CLEVELAND, OH  
**Project Period:** 2004/06/01-2006/05/31

DESCRIPTION (provided by applicant): Anthrax is an acute disease caused by the gram-positive bacterium *Bacillus anthracis*. Among the primary cellular targets are mononuclear phagocytes, which undergo both apoptosis and necrosis as a result of exposure to toxins produced by *Bacillus anthracis*. Lethal toxin (LT) is composed of two protein subunits, Protective antigen (PA) and Lethal Factor (LF). PA is a membrane-integrating protein that facilitates LF entry into cells. LF is a metalloprotease that specifically cleaves the NH2 termini of MAP kinase kinases (MAPKKs). The consequential inhibition of the activation of MAP kinases is thought to be central to *Bacillus anthracis*' evasion of the innate immune response. We present evidence that treatment of macrophages with interferon beta (IFNbeta) or interferon gamma (IFNgamma) promotes survival of macrophages after LT exposure. Although IFNbeta or IFNgamma does not protect macrophages from LT induced cleavage of MAPKKs, it does protect cells from cleavage of two important protein tyrosine phosphatases (SHP-1 and SHP-2), that have not been previously identified as substrates for LT. These findings suggest that interferons may provide a potential therapeutic approach to inhibit the biological actions of LT through prevention of the proteolysis of newly identified substrates that are targets of LT. We propose to: Aim 1: Identify proteins other than MAPKKs that are substrates for LT and determine which of these substrates are protected from LT mediated proteolysis in macrophages incubated with IFNbeta or IFNgamma. Aim 2: Determine the sites of cleavage of SHP-1 and SHP-2 as well as other proteins that are both proteolyzed by LT and are protected from proteolysis in cells exposed to LT and IFNgamma or IFbeta.

**Grant:** 1R21AI062275-01  
**Program Director:** NEAR, KAREN A.  
**Principal Investigator:** HUSSON, ROBERT N MD  
**Title:** M tuberculosis PknB: Targeting the Extracellular domain  
**Institution:** CHILDREN'S HOSPITAL (BOSTON) BOSTON, MA  
**Project Period:** 2004/07/01-2006/06/30

DESCRIPTION (provided by applicant): Tuberculosis (TB) remains a major cause of morbidity and mortality worldwide, particularly in much of the developing world. In regions where HIV and TB are co-prevalent, TB is a leading cause death in HIV-infected individuals. Where MDR-TB has emerged, including in the U.S., treatment is extremely difficult, mortality rates are high, and persons with HIV/AIDS have been disproportionately affected. New approaches to prevention and treatment, including new drugs that act through novel targets, are needed to treat drug-susceptible and MDR-TB more effectively. A signal transduction pathway mediated by the eukaryotic-like transmembrane serine/threonine kinases PknA and PknB is essential for the viability of *M. tuberculosis*. Data from the PI's laboratory and the *M. tuberculosis* genome sequence indicate that this signaling pathway regulates cell wall synthesis and cell division. The underlying hypothesis of this R21 proposal is that the extracellular domain of PknB (Ed-PknB) functions in vivo as a receptor domain or as a localization domain, through interaction with extracellular molecule(s), and that these interactions are essential for the function of PknB. The goals of this research are to gain insight into the PknA/B signaling network through identification and characterization of synthetic ligands of ED-PknB, and to determine whether such ligands have potential as leads for novel anti-tuberculars. These goals will be addressed by the following specific aims and methods: 1) screening small molecule chemical libraries for compounds that bind to ED-PknB using a novel glass slide microarray technology, validating positives and quantifying the strength of interactions by surface plasmon resonance, and 2) investigating the effects of the interaction of these ligands, and active derivatives, on mycobacterial viability and cell morphology, and on PknB kinase activity. The long-term goals of this research are to gain insight into the PknA/B signaling pathways, and to determine whether synthetic ligands of ED-PknB have potential as leads for new approaches to the treatment or prevention of TB.



**Grant:** 1R21AI062332-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** HO, JOHN L MD  
**Title:** Antigen Capture Assays for Rapid Tuberculosis Diagnosis  
**Institution:** WEILL MEDICAL COLLEGE OF CORNELL UNIV NEW YORK, NY  
**Project Period:** 2004/08/01-2006/07/31

DESCRIPTION (provided by applicant): Mycobacterium tuberculosis (Mtb), the etiologic agent of tuberculosis (TB), has infected up to one-third of the world's population; the majority of these people reside in resource poor countries ravaged by HIV. TB is the leading cause of AIDS mortality in co-infected people. Rapid diagnosis and case identification are needed to control TB, minimize transmission, and improve AIDS survival. Over 90% of TB cases involve the lung and the diagnosis is made either by the microscopic examination of acid fast bacilli (AFB) strained sputum smears or culture. Cultures have a sensitivity of "70% but take several weeks for results. Smears have a lower sensitivity (approximately 45%) but can be done quickly. We have discovered a protein, CFP32, which is unique to the tuberculosis-causing organism, thereby has potential used as a diagnostic test. Based on a CFP32 "antigen capture assay", approximately 60% TB patients had the protein detected in their sputum while none of the patients with other lung diseases had CFP32 detected (100% specificity). Importantly, CFP32 was also detected in AFB smear and/or culture negative TB. Since M. tuberculosis is the only organism that produces CFP32, it was diagnostic of active pulmonary TB. Therefore, the "first generation" diagnostic assay developed using recombinant (r)CFP32 produced by E. coli was superior to sputum smear in sensitivity and approached the sensitivity of sputum culture. Although E. coli can produce large quantities of protein, these bacteria produce proteins that lack modifications introduced by tuberculosis-causing bacteria. The use of E. coli expressed CFP32 to produce antisera likely resulted in the "first generation" CFP32 capture assay having a lower sensitivity than its true potential. Therefore, this grant seeks to obtain rCFP32 expressed in genetic systems that closely approximate M. tuberculosis to immunize animals and to generate several high affinity antibodies in rabbit and mouse for a second generation CFP32 capture assay that can better detect the native protein in clinical samples. In addition, detection of other highly secreted mycobacteria protein, Ag85 complex proteins, will be evaluated as co-antigens using existing reagents. The predictive value of these "second generation" assays will be established using sputum samples from "suspected" TB patients (200 HIV-seronegative, 50 HIV-seropositive). The proposal will evaluate the sensitivity of the combined CFP32/Ag85 antigen capture assays, compare the sensitivity of AFB smear and culture in the same sample, and determine the ability of the new assay(s) to diagnosis TB in smear and culture negative cases.

**Grant:** 1R21AI062541-01  
**Program Director:** LAMBROS, CHRIS  
**Principal Investigator:** RYAN, ROBERT O PHD  
**Title:** Amphotericin B Nanodisks and Cryptococcal Meningitis  
**Institution:** CHILDREN'S HOSPITAL & RES CTR AT OAKLAND, CA  
OAKLAND  
**Project Period:** 2004/09/30-2006/08/31

DESCRIPTION (provided by applicant): The long-term goal of our research is to improve therapy options for individuals suffering from systemic fungal infections. HIV infected individuals are at increased risk for opportunistic fungal infection and current treatment regimens are not optimal. Infection with *Cryptococcus neoformans* represents a serious problem that results in the potentially fatal disease, cryptococcal meningitis. The antibiotic, amphotericin B (ampB) is active against *C. neoformans* but serious side effects limit its use. By the same token formulation of this insoluble antibiotic with lipids decreases toxic side effects of the drug, permitting higher doses to be administered. Currently, three different lipid formulations of ampB are approved by the FDA for treatment of systemic fungal infections that are refractory to standard ampB deoxycholate (Fungizone) therapy. During the course of studies of the lipid interaction properties of a unique family of proteins, the amphipathic apolipoproteins, we discovered a method to incorporate ampB into discrete, lipid protein particles, termed Nanodisks. AmpB-Nanodisks possess up to 30 % of their lipid mass as ampB and exist as a homogenous population of disk-shaped particles wherein a phospholipid bilayer containing the ampB is circumscribed by apolipoprotein molecules around the perimeter of the disk. Characterization studies revealed that ampB Nanodisks are stable entities and may be lyophilized and reconstituted without loss of structural integrity. In vitro growth inhibition assays with various pathogenic fungal species revealed that ampB-Nanodisks inhibit 90 % of fungal growth as concentrations far lower than the liposomal formulation of ampB, AmBisome. In the present proposal we plan to optimize the composition, structure, and stability of ampB- Nanodisks, evaluate hypotheses related to the mechanism of the observed enhanced biological activity and determine the in vivo efficacy of ampB-Nanodisks in an animal model of cryptococcosis. We anticipate the results obtained will lead to new treatment options for AIDS related cryptococcal meningitis that offer advantages over existing therapies.

**Grant:** 2R37AI014937-26  
**Program Director:** PETERS, N KENT  
**Principal Investigator:** TOWNSEND, CRAIG A PHD CHEMISTRY:ORGANIC  
**Title:** Biosynthesis of Beta-Lactam Antibiotics  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 1978/09/01-2009/03/31

Penicillin and related beta-lactam antibiotics have been a mainstay in the treatment of infections for 50 years. Their effectiveness, however, like other known classes of antibiotics has come under increasing challenge from the rise of multiply drug-resistant pathogenic bacteria. Efforts have intensified to understand the mechanisms of resistance and to overcome them. Structural modification through genetic manipulation of their biosynthetic pathways is a promising approach to produce variants of known antibiotics by cost-effective fermentation and semi-synthetic methods. Continuation of a program to investigate beta-lactam antibiotic biosynthesis is proposed in this application. Three of the four known classes of these antibiotics will be studied: (1) clavulanic acid, a potent inhibitor/inactivator of beta-lactamase enzymes and a wide-spread source of resistance, (2) the nocardicins, a family of monocyclic beta-lactams, and the metabolically related monobactams, and (3) the carbapenems, represented clinically by thienamycin and its derivatives, but most simply by carbapen-2-em-3-carboxylic acid. Characterization of the biosynthetic gene clusters for at least one member of each of these principal groups has led to rapid advances in the current grant period using techniques ranging from organic synthesis and enzymology to molecular biology and macromolecular structural methods. It is proposed to pursue these discoveries through mechanistic and structural studies of N<sup>2</sup>-(carboxyethyl)-L-arginine synthase, site-specific mutagenesis to examine the mechanism and engineer the function of beta-lactam synthetase, collaborative studies to characterize the iron center of clavaminic synthase, and substrate analogue and site-directed mutagenesis experiments to examine its mechanism, and to investigate the "enantiomerization" that occurs in the penultimate step of clavulanic acid biosynthesis. Disruption and over-expression of nocardicin biosynthetic genes will be undertaken to delineate the pathway, understand the mechanism of monocyclic beta-lactam formation and determine the roles of two unusual non-ribosomal peptide synthetases. Investigation of the biosynthetically related monobactams will be initiated. Characterization of three key proteins that form the carbapenem nucleus will figure prominently in studies of this group, and new experiments with thienamycin will be begun to understand the more complex members of this family and engineering of their synthesis.

**Grant:** 2R37AI021657-19

**Program Director:** SCHMITT, CLARE K.

**Principal Investigator:** KAPER, JAMES B PHD  
MICROBIOLOGY:BACTERIOLOG  
Y

**Title:** Molecular Genetics of Enteropathogenic E. Coli Adhesion

**Institution:** UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD  
SCHOOL

**Project Period:** 1986/07/01-2009/03/31

DESCRIPTION (provided by applicant): Enteropathogenic Escherichia coli (EPEC) are an important cause of diarrhea in infants. The long-term objectives of this project are to understand the pathogenesis of disease due to this organism. Previous work on this project has resulted in the discovery of a 35 kb pathogenicity island called the Locus of Enterocyte Effacement (LEE) that is responsible for the attaching and effacing (AE) histopathology on intestinal epithelial cells that is the hallmark of EPEC infections. The LEE island encodes the epithelial cell adhesin named intimin, a type III protein secretion system, and several effector proteins that are translocated into epithelial cells to mediate the AE lesion. Typical EPEC strains possess the LEE plus the EAF plasmid that encodes a type IV pilus (BFP) that contributes to epithelial cell adherence and a positive regulator of EPEC virulence factors called Per. Typical EPEC strains are an important cause of infant diarrhea in developing countries but are infrequent causes of disease in the U.S. Atypical EPEC strains contain the LEE but lack the EAF plasmid. In contrast to typical EPEC strains, atypical EPEC appear to cause outbreaks and sporadic disease in adults in industrialized countries such as the U.S., Finland, and Japan. Recent studies of atypical EPEC in children with diarrhea in the U.S. suggest that the incidence of infections with this pathogen may equal or exceed that of Salmonella, Shigella, Campylobacter, or E. coli O157:H7. The proposed research goals for the next period of support include continued characterization of pathogenic mechanisms of typical EPEC strains, particularly genes encoded outside the LEE, and a new focus on potential pathogenic mechanisms of atypical EPEC. The specific aims are 1) Characterize the Long Polar Fimbriae (LPF) of EPEC and assess in vivo expression of this and other potential adhesins; 2) Identify and characterize genes regulated by the Ler and Per regulators of EPEC virulence factors using genomic arrays; 3) Characterize human host epithelial cell transcriptional responses to EPEC infection using in vitro organ culture (IVOC); and 4) Characterize potential virulence factors in atypical EPEC strains.

**Grant:** 2R37AI026170-16  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** JACOBS, WILLIAM R PHD  
**Title:** Molecular Genetic Analysis of Mycobacterium tuberculosis  
**Institution:** YESHIVA UNIVERSITY BRONX, NY  
**Project Period:** 1988/12/01-2009/01/31

DESCRIPTION (provided by applicant): Tuberculosis (TB) is the leading cause of adult death by a single infectious organism, accounting for over 2.3 million deaths per year worldwide. The emergence of drug resistance and the increased spread of TB promoted by the global epidemic of HIV are worsening the global tuberculosis problem. Clearly, novel strategies are needed to control the tuberculosis epidemic. The acquisition of basic knowledge of the causative agent of TB, Mycobacterium tuberculosis, is a prerequisite for developing such tools. The goal of this proposal has been to develop the set of tools necessary for the generation of mutants and the transfer of genes M. tuberculosis mutants. Using mycobacteriophages as a starting point, we have developed the first transformation systems for mycobacteria, the ability to generate random mutants with transposons, and, recently, the ability to efficiently generate targeted gene disruptions using specialized transduction. The combination of these tools with the acquisition of the genomic sequence of M. tuberculosis provides unprecedented opportunities to acquire basic knowledge of the tubercle bacillus. Indeed, our successes in developing these tools have allowed us to: i) determine the previously unknown targets of the anti-TB drugs isoniazid and ethionamide, ii) identify numerous novel genes required for M. tuberculosis virulence, and iii) to determine the genetic and functional basis for the attenuation of the TB vaccine strain, BCG. This proposal seeks to use these genetic systems in combinations with a number of newly developed screens for mutants defective in virulence traits. The tubercle bacillus has acquired the means to invade and multiply in macrophages. Moreover, M. tuberculosis has evolved mechanisms by which it overcomes effector mechanisms of the host's innate and adaptive immune responses. We plan to elucidate the mechanisms by which M. tuberculosis causes pathogenesis and overcomes the innate and adaptive immune responses of the host by screening for mutants that are defective in their abilities to: i) grow in mammalian lungs, ii) to modulate different cytokine responses, and iii) to grow and persist in immunocompetent and immunocompromised mice. Ultimately, the knowledge of mycobacterial virulence and persistence factors should lead to the development of novel drugs, vaccines, and immunotherapies to control tuberculosis.

<b>Grant:</b>	4R37AI029549-14	
<b>Program Director:</b>	KORPELA, JUKKA K.	
<b>Principal Investigator:</b>	HULTGREN, SCOTT J	PHD MICROBIOLOGY MOLECULAR BIOLOGY
<b>Title:</b>	CHAPERONE ASSISTED PILI ASSEMBLY IN PATHOGENIC E COLI	
<b>Institution:</b>	WASHINGTON UNIVERSITY	ST LOUIS, MO
<b>Project Period:</b>	1991/03/01-2009/03/31	

Abstract Text Not Available

**Grant:** 2R37AI032725-11

**Program Director:** QUACKENBUSH, ROBERT L.

**Principal Investigator:** RICE, PETER A MD INTERNAL  
MED:INFECTIOUS DISEASES

**Title:** Immunology of Infection with *Neisseria gonorrhoeae*

**Institution:** BOSTON MEDICAL CENTER BOSTON, MA

**Project Period:** 1993/01/01-2009/03/31

DESCRIPTION (provided by applicant): *Neisseria gonorrhoeae* is one of the two major pathogens involved in the majority of cases of sexually transmitted genital infection. Complement forms an important aspect of the innate immune system that impacts upon gonococcal infection. Prior work in our laboratory has shown that sialylation of gonococcal lipooligosaccharide (LOS) results in complement resistance by binding the host complement regulatory molecule, factor H. The porin molecule also binds factor H. In the first Specific Aim, we will investigate the role of an alternatively-spliced version of factor H, called factor H-like molecule 1 (FHL-1) in binding to gonococci and in regulating complement. Some strains of *N. gonorrhoeae* process complement (i.e. convert complement component-3 [C3b] to the inactivated form [iC3b]) and bind FHL-1, but not factor H. Cofactor activity of FHL-1 will be assessed using serum containing only FHL-1, but not intact factor H. Because both factor H and FHL-1 bind to cells, we will examine the roles of these two molecules in facilitating gonococcal attachment to immortalized cervical and urethral epithelial cells. In the second Specific Aim, three questions that pertain to LOS sialylation will be addressed. First, the specificity of factor H binding to gonococcal lacto-N-neotetraose (LNT) sialic acid, but not to meningococcal LNT sialic acid, will be examined. Porin (Por) influences binding of fH to gonococcal sialic acid. We will perform allelic exchange of porin molecules between the two species to examine the effect of porin on the sialylated LOS interactions with factor H that differs at baseline in the two neisserial species. Second, we will examine the determinants of the functional specificity of the LOS sialyl transferase (Lst) enzyme in meningococci and gonococci by performing allelic exchanges of the Lst genes between the two species. Third, we will also determine why the efficiency of LOS sialylation differs between serum-sensitive (high sialic acid uptake) and "stably" serum-resistant (low sialic acid uptake) gonococci. This will be performed by examining the uptake of <sup>3</sup>H-labeled CMP-NANA by isogenic gonococci differing only in their Lst enzymes. The possibility that Por modifies Lst activity will also be examined by performing allelic exchanges of the Por genes between high and low sialic acid incorporators. In the third Specific Aim, we will detail the linkages (amide versus ester) between C4 and LOS, the effects of hexose extension of the LOS on binding of C4 to LOS, and the impact of the bond formed between C4 and LOS on bactericidal killing.

**Grant:** 1U01AI060594-01  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** BERGERON, MICHEL G. MD  
**Title:** Microfluidic system for point of care diagnosis of GBS  
**Institution:** LAVAL UNIVERSITY QUEBEC, CANADA G1V 4G2, PQ  
**Project Period:** 2004/09/01-2009/08/31

**DESCRIPTION** (provided by applicant): This multidisciplinary collaborative research project between investigators of the University of Laval and Infectio Diagnostic (I.D.I.) Inc. in Quebec City and the University of California at Irvine, aims at developing novel, flexible, and rapid diagnostic devices based on nucleic acid testing. The pinnacle in molecular diagnostics is to have a disposable point-of-care handheld device combining nucleic acid extraction, concentration, purification, amplification, and detection to specifically and sensitively detect the target sequences. Such devices would allow the integration of diagnosis and therapy as well as development of personalized medicine. Technologies based on nucleic acid detection that are currently available or under development are slow, complicated, insufficiently sensitive, and expensive. The objective of this application is to merge our expertise in microfluidics and polymeric transducers to design and construct diagnostic devices of the future which do not require prior nucleic acid amplification. A rapid molecular assay for the specific and sensitive detection of Group B Streptococci (GBS) colonization in pregnant women at delivery will be developed. GBS are an important cause of neonatal morbidity and mortality. A portable micro total analysis system ( $\mu$ /TAS) using a microfluidic platform on a compact disc support will be developed. It will be compact, battery operated and its simple utilization and low cost will make it suitable for testing at a field site, in a clinic, or in hospitals. This novel diagnostic platform will be optimized for GBS detection from vaginal/anal specimens. It will be validated by a pre-clinical study with samples obtained from consenting pregnant women at delivery that will be performed in collaboration with several hospitals. This new technological platform should provide unprecedented point-of-care diagnostic tools for GBS, permitting a better control of antibiotic use, and reducing dissemination of resistant microbes. Moreover, this technology could also be applicable to the detection of other infectious disease agents including biological warfare agents.



**Grant:** 1U01AI060595-01  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** MUSSER, JAMES MALLORY MD  
**Title:** NOVEL GROUP A STREPTOCOCCUS HUMAN VACCINE CANDIDATES  
**Institution:** BAYLOR COLLEGE OF MEDICINE HOUSTON, TX  
**Project Period:** 2004/08/01-2009/07/31

DESCRIPTION (provided by applicant): This study is designed to identify one or more conserved group A Streptococcus (GAS) proteins suitable for use as a safe and efficacious human vaccine worldwide. The key goal of the proposed research is to use genome-wide methods of contemporary vaccinology to identify one or more conserved GAS proteins that will significantly protect monkeys from pharyngitis caused by challenge with a strain expressing a heterologous M protein serotype. A conservative estimated timeline is that by the end of the 5-year research period, the antigen(s) will be ready for detailed toxicity testing prior to beginning phase I human trials. The international investigative team composed of academic and pharmaceutical industry collaborators proposes the following line of research involving a "reverse vaccinology" strategy: Aim 1: Use two mouse models of invasive GAS disease to confirm extensive preliminary data that novel candidate GAS antigens significantly protect immunized mice challenged with a GAS strain expressing a heterologous M protein serotype. Aim 2: Determine if the proteins satisfying the mouse screen criteria described in aim (1) above are conserved in natural populations of GAS, expressed on the cell surface of genetically diverse GAS strains, and expressed in vivo during diverse types of human infections (pharyngitis, invasive infections, etc). Aim 3: Use a recently-described monkey model that mimics human pharyngitis to determine if one or more of the candidate vaccine antigens we identify in aim (1) and (2) significantly protects against pharyngitis caused by a GAS strain expressing a heterologous M protein serotype.

**Grant:** 1U01AI060603-01  
**Program Director:** RUBIN, FRAN A.  
**Principal Investigator:** PAOLETTI, LAWRENCE C PHD  
**Title:** Development of a Global GBS Vaccine  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 2004/07/15-2008/06/30

DESCRIPTION (provided by applicant): Group B Streptococcus (GBS) is an important human pathogen. At-risk populations include babies born to colonized mothers, peripartum women, diabetics, and the elderly with underlying illnesses. Vaccines to prevent GBS disease have been developed by coupling capsular polysaccharide (CPS) antigens of GBS to immunogenic protein carriers. Glycoconjugate vaccines against all nine GBS serotypes have been synthesized and shown to be immunogenic in animals in preclinical trials. Healthy adults have safely received conjugate vaccines prepared with GBS types Ia, Ib, II, III, and V CPSs in phase 1/2 clinical trials. These vaccines elicited CPS-specific antibody that opsonized GBS for in vitro killing by human blood leukocytes in the presence of complement. Despite these advances, a GBS vaccine for public use has not been developed due to the number of components required and the shifting pattern of serotypes in the population. Advances in vaccine development has been accelerated by the sequencing of the GBS genome and new protein antigens have been revealed using reverse vaccinology. This proven approach to vaccine development promises to be applicable to GBS vaccines. In this application, we propose to develop a combination vaccine composed of 3 to 4 recently identified protective proteins combined with GBS glycoconjugates representing the three major disease causing serotypes which will be effective against the large majority of GBS variants of all GBS serotypes. A systematic approach will: a) identify, express and purify GBS proteins, b) assess the conservation of these proteins among GBS strains, c) synthesize and test conjugate vaccines in animals using the new GBS antigens as carrier proteins, d) formulate and determine the efficacy of a multivalent GBS vaccine. Successful completion of these objectives will provide the rationale for: e) preparation of a multivalent GBS vaccine made under cGMP, and f) phase 1/2 clinical trials of a multivalent GBS vaccine in healthy nonpregnant (18 to 65 years of age) and elderly (>65 years of age) adults. This work will lead to a vaccine effective in preventing GBS diseases worldwide.

**Grant:** 1U01AI061187-01  
**Program Director:** HALL, ROBERT H.  
**Principal Investigator:** WEIGL, BERNHARD H. PHD  
**Title:** A Multiplex, Point-of-Care Test for Enteric Pathogens  
**Institution:** PATH - PROG/APPROPRIATE TCHNLGY IN SEATTLE, WA  
HLTH  
**Project Period:** 2004/09/01-2008/08/31

**DESCRIPTION** (provided by applicant): The overall goal of the proposed research is to adapt and apply an existing nucleic acid (NA) amplification, lab-on-a-card platform to identify enteric bacteria in patients presenting to primary health care settings or public health laboratories with symptoms of acute diarrhea. Component functions to be integrated on this platform include multiplex NA amplification and detection, sample processing to support direct use of clinical specimens, and dry reagent storage and handling. This new, robust, diagnostic test will provide a rapid, highly sensitive, specific, easy-to-use, cost-effective tool to distinguish bacteria likely used in bioterrorism attacks from bacteria that cause similar generalized symptoms. The four-year project is designed to achieve the following activities and milestones: 1. Develop immunological approaches to increase specificity and enable direct use of fresh stool or rectal swab specimens. 2. Develop a multiplex NA amplification, lab-on-card platform for rapid identification of *Shigella dysenteriae*, *Shigella toxin-producing Escherichia coli*, *Campylobacter jejuni*, and *Salmonella* species simultaneously from the same clinical specimen. 3. Optimize on-board reagent storage and handling capabilities. 4. Integrate reagent storage and handling, sample processing, and NA amplification and detection onto a single, lab-on-a-card, disposable enterics card system. 5. Collect clinical specimens to support research and development efforts and assessment of the clinical sensitivity and specificity of the assay. A consortium agreement among Program for Appropriate Technology in Health (PATH), Seattle, WA; Micronics Inc., Redmond, WA; Xtrana Inc. (formerly Biopool Inc.), Broomfield, CO; the University of Washington, Seattle, WA; and Washington University, St. Louis, MO, will provide for a world-class, multidisciplinary team skilled in microbiology; biochemistry; genomics; microfabrication; product development and scale-up of high-volume, regulated, disposable devices and instruments; DNA molecular physics; dry chemistry stabilization; clinical research; and overall technical management.

**Grant:** 1U01AI061192-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** CUNNINGHAM, PHILIP R PHD  
**Title:** Anti-infectives that target bacterial ribosomes  
**Institution:** WAYNE STATE UNIVERSITY DETROIT, MI  
**Project Period:** 2004/07/15-2008/06/30

DESCRIPTION (provided by applicant): Bacteria have developed resistance to all of the antibiotics currently in use. A major concern regarding the use of bacterial pathogens as biological weapons is the possibility that they have been engineered to be resistant to current chemotherapeutic agents. The goal of this project is to develop new classes of anti-infectives that are not susceptible to natural or engineered resistance mechanisms. The bacterial ribosome is a proven drug target. We have developed new genetic technology ('instant evolution') that allows high-throughput in vivo isolation and analysis of ribosomal RNA mutations that might lead to drug resistance. This information will be used to identify anti-infectives that recognize the wild-type and all viable mutants of the drug target. Instant evolution was initially developed using *Escherichia coli*, but was recently applied to the rRNA genes of several other major human pathogens including *Yersinia* species. The proposed multidisciplinary project brings four groups together with expertise microbial genetics, structural biology, in vitro selections, organic synthesis, drug screening, and combinatorial chemistry to create a unique collaborative team focused on the development of entirely new classes of antimicrobial therapeutics. Antimicrobials produced using this platform will provide new therapies for the treatment of infections caused by human pathogens that are resistant to current antibiotics. In addition, the new therapeutics will be less susceptible to de novo development of resistance because instant evolution allows all mutations of the target that might lead to resistance to be identified during the earliest stages of the drug discovery process.

**Grant:** 1U01AI061199-01  
**Program Director:** ZOU, LANLING  
**Principal Investigator:** DUBENSKY, THOMAS W PHD  
**Title:** Psoralen-Killed, Metabolically-Active Anthrax Vaccine  
**Institution:** CERUS CORPORATION CONCORD, CA  
**Project Period:** 2004/07/01-2007/06/30

DESCRIPTION (provided by applicant): The only licensed human anthrax vaccine, anthrax vaccine absorbed (AVA), was developed in the late 1950s and is poorly immunogenic. The prolonged 18-month vaccination regimen and required annual boosters are especially problematic for immunization of military personnel both in terms of safety and in terms of practicality. The possibility of new virulent strains that have been strategically engineered to subvert the limited immune response elicited by the AVA vaccine constitutes a genuine threat. Cerus has developed a novel approach for clinically safe and potent vaccines against microbial pathogens utilizing its technology based on the proprietary S-59 psoralen. The S-59 psoralen (S-59) is approved for human use in Europe as part of the commercially available INTERCEPT pathogen inactivation system for platelets. As a proof of concept, mutant strains of the human pathogen *Listeria monocytogenes* were created by deleting the *uvrAB* genes. This deletion rendered the DNA repair mutant bacteria exquisitely sensitive to S-59/UVA light-mediated inactivation, but preserved metabolic activity and expression of the *Listeria* genetic repertoire. As a result, S-59/UVA-inactivated *Listeria uvrAB*-induced protective memory T cell responses and significant antibody responses in vaccinated animals. With this application, we propose to construct a panel of S-59/UVA inactivated anthrax vaccine candidates based on nonsporogenic *B. anthracis* DNA repair mutants utilizing host strains having both pXO1 and pXO2 virulence plasmids. The primary goal of this proposal is to identify a vaccine candidate for further testing in nonhuman primates. A series of experiments to be performed in both mice and guinea pigs are proposed to identify an optimal anthrax vaccine candidate based on safety, combined with immunogenicity and induction of protection against lethal toxin or spore challenge. We hypothesize that this unique approach of combining non-viability with metabolic activity within the context of the whole *B. anthracis* organism will result in a vaccine that induces protective bacterial-specific immunity with increased depth (i.e. mucosal, humoral, and cellular immunity), breadth (immune response targeted at multiple bacterial antigens including capsule), and durability (long-term immunological memory) with a practical immunization regimen, as compared to the AVA vaccine. Achieving the primary goal set forth in this proposal will set the stage for longer-term objectives of the program, and for moving the project toward an initial human clinical trial to test the immunogenicity and safety of this platform. The ultimate goal of this work is to replace the current AVA human anthrax vaccine with an S-59 psoralen/UVA light inactivated genetically defined attenuated nonsporogenic strain of *B. anthracis*. To accomplish this goal to develop what is a new class of vaccines that combine the safety of a killed vaccine with the potency of a live-attenuated vaccine, we have assembled an experienced consortium comprised of scientists with documented expertise in bacterial pathogenesis, Gram-positive genetics, novel vaccine platform development, infectious disease vaccine development, and a history of productive collaboration.

**Grant:** 1U01AI061271-01  
**Program Director:** ZOU, LANLING  
**Principal Investigator:** JANDA, KIM D  
**Title:** Human Monoclonal IgG for Protection against Anthrax  
**Institution:** SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA  
**Project Period:** 2004/06/01-2007/05/31

DESCRIPTION (provided by applicant): Passive immunization with human monoclonal antibodies (mAbs) against anthrax could be used by military personnel during warfare or the general public in the event of bioterrorist activity. To establish a foundation for potential clinical testing, we propose to develop and characterize human mAbs in the IgG format against both spores and the protective antigen (PA) of *B. anthracis*. Key features of the research include our 1) phage-display human scFv libraries, 2) experience in obtaining scFvs against *Bacillus* spores and various protein antigens, 3) expertise in molecular evolution to enhance scFv affinity, and 4) ability to convert scFvs into the Fab and whole human IgG formats. Our strategy: 1) scFvs will be obtained against spores by biopanning, selection, and characterization; 2) conversion of the best anti-spore scFvs to the Fab format for retesting, and assembly of human IgGs; 3) scFvs will be obtained against PA not only by conventional biopanning, but also by whole-cell biopanning of PA in the active, membrane-associated structure; 4) conversion of the best anti-PA scFvs to the Fab format for in vitro assays, affinity-enhancement and retesting, and assembly of human IgGs; 5) final testing of anti-PA IgGs in vitro for protection against cytotoxicity from exotoxin (PA in combination with lethal factor, LF), and studies of both anti-spore and anti-PA IgGs with regard to macrophage-spore-IgG interactions. We believe all experiments, both established and novel, will provide valuable information about the future potential of passive immunization as a treatment and/or prophylaxis for anthrax.

**Grant:** 1U01AI061297-01  
**Program Director:** BEANAN, MAUREEN J.  
**Principal Investigator:** WOLINSKY, STEVEN M MD OTHER AREAS  
**Title:** Detection of Category A Pathogens by Gold Nanoparticles  
**Institution:** NORTHWESTERN UNIVERSITY EVANSTON, IL  
**Project Period:** 2004/09/30-2008/08/31

DESCRIPTION (provided by applicant): Recent deliberate exposure of civilian population of the United States populace to *Bacillus anthracis* spores uncovered an unmet need for tests to rapidly diagnose disease caused by this bacterium. A major challenge for rapid and accurate pathogen detection is the development of methods that do not rely on the polymerase chain reaction (PCR) or comparable target- or signal-amplification systems to minimize instrumentation and reagent needs and enable point-of-care or field testing. We propose to develop a highly selective sequence-specific, chip-based method for detection of DNA from *B. anthracis* by exploiting the unique properties of DNA-gold nanoparticle conjugates. The proposed methodology promises to be far simpler than current methods and obviates the need for large-scale analytical instrumentation and trained personnel. Preliminary data show that detection assays based on these novel nanostructures are substantially more sensitive and selective than conventional assays based on fluorophore-labeled DNA probes. Ultimately, the assay will be as accurate and sensitive as PCR-based assays. By combining gold nanoparticles with Raman spectroscopic fingerprints, we can effectively distinguish dissimilar polynucleotide sequences of multiple infectious pathogens in the same sample without target sequence amplification. In contrast to fluorescence-based hybridization, gold nanoparticle probes labeled with oligonucleotide and Raman-active dyes offer several advantages, including higher potential for multiplexing and orders-of-magnitude higher sensitivity and selectivity. We propose to implement this assay in a simple microfluidic device with integrated capacity for specimen processing, DNA/RNA purification, separation, hybridization, and signal detection, thus offering the possibility for integrated, high-throughput screening in a "lab-on-a-chip," with easily-read outputs. The overall goal of this proposal is to create a rapid, sensitive, specific, and cost-effective tool for detection and identification of the category A pathogen, *B. anthracis*, and a battery of other pathogens with similar clinical syndromic presentation using a single sample. In Specific Aim 1, we will develop a rapid, high-sensitivity, high-selectivity sequence-specific test based on the optical, hybridization, and catalytic properties of gold nanoparticle DNA conjugates and Raman-active dyes for the simultaneous detection of multiple target polynucleotide molecules. In Specific Aim 2, we will design, develop, and fabricate a flexible polynucleotide array-based microfluidic platform with cell and analyte handling capabilities for cost-effective, high-throughput, selective detection of captured target DNA and RNA bound to nanoparticles capable of surface-enhanced Raman scattering (SERS). Lastly, in Specific Aim 3, we will establish proof-of-principle and validate the performance of the array-based multiplex detection of conserved polynucleotides derived from anthrax and other infectious pathogens with a similar respiratory syndromic presentation at the point-of-care.

**Grant:** 1U01AI061311-01  
**Program Director:** ZOU, LANLING  
**Principal Investigator:** BOWDISH, KATHERINE S BS  
**Title:** Human Antibody Therapeutics Against Anthrax  
**Institution:** ALEXION ANTIBODY TECHNOLOGIES, INC. SAN DIEGO, CA  
**Project Period:** 2004/07/15-2007/06/30

**DESCRIPTION** (provided by applicant): The long-term objective of our research is to develop fully human antibodies as immunotherapeutics against anthrax infection in humans. Mab 83K7C and Mab 63LID are two lead candidates identified using phage display technology that protect rats in vivo against a lethal anthrax toxin challenge. These two antibodies neutralize potently via differing mechanisms. In order to determine efficacy against an inhalational anthrax infection and possibly discriminate between these candidates so as to move at least one toward therapeutic use, this application is specifically targeted to the performance of preclinical pharmacokinetic and spore challenge studies in rabbits and monkeys. If protection is shown, these studies will be used to begin to fulfill the requirements for the two animal models requested by the FDA for biodefense therapeutics that cannot undergo Phase II and Phase III clinical testing in humans for ethical reasons (21CFR 314.610 and 601.91). The project has been broken down into five specific aims. 1) Sufficient antibody must be produced and purified for the trials under consideration, and this will be performed at Kemp Biotechnologies, Inc. (Frederick, MD). Material will be analyzed and trial dosages prepared and validated by applicant. 2) Pharmacokinetic trials in rabbits will be performed at Charles River Laboratories (CRL) (Worcester, MA) to help establish appropriate dosing and route of administration for spore challenge trials. 3) Rabbit spore challenge trials will be performed at Battelle Medical Research and Evaluation Facility (MREF) (Jefferson, OH). 4) If efficacy in rabbits is demonstrated, monkey pharmacokinetic trials with one or both antibodies will be performed at CRL. 5) Spore challenge trials in monkeys will be performed at Battelle MREF. GLP conditions will be used in production and for both the pharmacokinetic and spore challenge studies such that data obtained from these trials could be acceptable for FDA submittal. If spore challenge trials in monkeys are successful, we will continue with additional preclinical and Phase I human trials outside the scope of this grant.



**Grant:** 1U01AI061314-01  
**Program Director:** ZOU, LANLING  
**Principal Investigator:** LOWY, ISRAEL AB  
**Title:** Development of Fully Human mAbs as Anthrax Antitoxins  
**Institution:** MEDAREX, INC. BLOOMSBURY, NJ  
**Project Period:** 2004/09/15-2007/08/31

**DESCRIPTION** (provided by applicant): This grant proposes to investigate the potential utility and mechanism of action of fully human monoclonal antibodies (HuMAbs) to the anthrax protective antigen (PA) as prophylactic and therapeutic antitoxins. For immunization, Medarex, Inc. uses its proprietary HuMab mice, which express transgenic human antibody genes and have an inactivated murine antibody locus. These mice produce fully human, affinity-matured antibodies, which are isolated and produced by hybridoma and transfectoma technology. Under a CRADA with USAMRIID and Dartmouth University, Medarex, Inc. has developed a panel of HuMAbs that react with and potentially neutralize Anthrax toxin in in vitro cytotoxicity assays, have defined a novel neutralizing epitope on PA-63, and provide protection and therapy in an inhalational anthrax rabbit model. From this panel, 1 monoclonal antibody (mAb 5E8) and 1 backup antibody (5D5) were selected for further development. The HuMAbs were selected based on superior anthrax toxin neutralizing activity in macrophages. The HuMab 5E8 affords extended survival to rabbits after inhalation of a lethal dose of anthrax spores, when administered concurrently with the exposure (prophylaxis / post-exposure prophylaxis activity), and even when given after the onset of clinical signs of anthrax disease (therapeutic activity). The envisioned practical usage of this antibody will be to provide rapid passive immunity to individuals at risk for exposure to *Bacillus anthracis* and ensuing serious disease. The usage may be prophylactic in pre- or post-exposure situations, but more importantly, may have efficacy in treatment of individuals with active disease for which there is no current effective therapy. To further investigate the utility and mechanisms of action of this antibody, Medarex, Inc. aims to: 1. Further define the specific mechanism(s) of protection conferred by HuMab 5E8 and other anti-PA monoclonal antibodies; 2. Develop current animal models of inhalation anthrax amenable for the investigation of therapeutic activity; 3. Investigate the activity of HuMab 5E8 in therapeutic models of inhalation anthrax; and 4. Investigate the effect of the antibody on the development of natural immunity to infection and on the immunogenicity of the PA vaccine.

**Grant:** 1U01AI061336-01  
**Program Director:** LAUGHON, BARBARA E.  
**Principal Investigator:** EDWARDS, DAVID A  
**Title:** Inhaled Large Porous Particles for Treatment of MDR-TB  
**Institution:** HARVARD UNIVERSITY CAMBRIDGE, MA  
**Project Period:** 2004/07/01-2007/06/30

DESCRIPTION (provided by applicant): We seek to develop an aerosol delivery approach to more effectively treat and improve the control over transmission and outbreak of respiratory infectious diseases, specifically tuberculosis (TB) and multi-drug resistant TB (MDR-TB). Our hypothesis is that direct, topical delivery of antibiotics to infected lungs results in relatively high local drug concentrations, which can more quickly eradicate active bacterial populations, thus sterilizing the lungs and reducing the duration of infectivity and the duration of chemotherapy necessary to achieve a durable cure in pulmonary tuberculosis relative to parenteral or oral dosing. We propose to develop and test anti-TB agents and combinations that can be administered directly to patient lungs from an inexpensive, easy-to-use inhaler suitable and appropriate for use even in resource-limited settings. The agents will be formulated into large porous particles (LPPs) whose unique physical characteristics enable highly efficient delivery of relatively large drug masses. This approach has the added advantages of being non-invasive and/ or reducing gastrointestinal and systemic side effects. Specific research aims are to develop stable, LPP formulations of key anti-TB agents, establish their efficacy in animal models of TB, complete 28-day toxicology testing, and to determine reliability in the field of an existing inhaler by challenging it harsh conditions. This work will result in the identification of lead candidate antibiotic aerosols suitable for further clinical development for the improved treatment of TB and MDR-TB. It represents a new paradigm for treating and limiting the spread (especially nosocomial transmission) of respiratory infectious diseases such as TB, severe acute respiratory syndrome (SARS), influenza, and small pox.

**Grant:** 1U01AI061363-01  
**Program Director:** SCHAEFER, MICHAEL R.  
**Principal Investigator:** FELGNER, PHILIP L PHD  
**Title:** Scanning *B. pseudomallei* proteome for vaccine antigens  
**Institution:** UNIVERSITY OF CALIFORNIA IRVINE IRVINE, CA  
**Project Period:** 2004/09/01-2009/08/31

DESCRIPTION (provided by applicant): Because of the potential bioterrorism threat, the development of a safe and effective *B. pseudomallei* vaccine is a national and worldwide goal. The Applied Proteomics Laboratory at UC Irvine has developed a high throughput protein expression system called PCR Express which can be used to rapidly generate complete proteomes from any sequenced microorganism, including the Class A, B & C Bioterrorism Agents. The technology allows hundreds of different genes to be expressed directly from their PCR products at the rate of hundreds of different proteins per week. Here the *Burkholderia pseudomallei* proteome will be generated and applied to the problem of identifying antigens recognized by B-cells and T-cells that will be useful in a subunit vaccine against the agent. The first form of the proteome will be on microarray chips which will be used to quantify serum antibody titers from BALB/c mice vaccinated with a rationally attenuated auxotroph of *B. pseudomallei* (2D2) and humans naturally exposed to *B. pseudomallei* in endemic regions of NE Thailand against each of the individual bacterial proteins. For the second proteome format, each individual protein will be purified and presented in a form that will enable them to be used in T cell restimulation assays in vitro using either spleen cells from 2D2 vaccinated mice or whole blood from exposed human populations. T cell activation will be determined by ELISA assay for secretion of IFN $\gamma$ . The responsive antigens identified by this Vaccine Antigen Scan will be tested for their efficacy in a murine model of infection with *B. pseudomallei* and, because of the extensive genetic similarity between *B. pseudomallei* and *B. mallei*, in animal models of glanders. This quantitative humoral and cellular immune response scan will produce the first complete profile of the immune response against *B. pseudomallei* in humans and experimental animals and will identify the most effective candidate antigens for development of a DNA or subunit vaccine against human melioidosis and glanders.

**Grant:** 2U19AI031448-14

**Program Director:** FRIEDMAN, HEIDI B.

**Principal Investigator:** HOLMES, KING K MD INTERNAL MED:INTERNAL  
MEDICINE-OTHER

**Title:** University of Washington STI-TM Cooperative Research Ctr

**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA

**Project Period:** 1997/07/01-2009/08/31

DESCRIPTION (provided by applicant): This UW STI TM CRC application includes 4 interrelated Projects, 2 scientific Cores (Biostatistics and Laboratory), and an STI TM CRC Developmental Awards Program. The First Project, Comparative Genomics of Clinical *C. trachomatis* Strains (Daniel Rockey) will use the Lab Core's large specimen repository to explore linkages of chlamydial genotypes to three unique phenotypes of *C. trachomatis*, seeking chlamydial genes important in previously uncharacterized aspects of chlamydial biology and pathogenesis. The Second Project, Immunogenetic Correlates of Severity of Genital HSV-2 Infection (Anna Wald) will identify MHC class I and II alleles associated with HSV-2 infection severity, and determine how HLA class I-restricted CMI responses exert selective pressures on HSV-2 amino acid sequences. The Third Project, Male Microbicide Safety Acceptance & Efficacy Against STI (King Holmes) plans preclinical studies, a Phase 1 trial, and a Phase 2/3 double-blinded placebo-controlled randomized controlled trial (RCT) in Kenya of 62% ethanol in emollient gel for preventing STI acquisition by men. This promising topical microbicide is nontoxic, preferred for hand hygiene in health care settings, widely available over-the-counter for domestic use, and has been well tolerated in our NIH-funded preparatory studies in Nairobi. The Fourth Project, *M. genitalium* Susceptibility and Treatment (Lisa Manhart) proposes development of innovative methods to measure antimicrobial susceptibility of *M. genitalium*, and a double blinded, placebo-controlled RCT to determine the relationship of clinical and microbiologic outcomes to drug used, and to *M. genitalium* drug susceptibility. Projects will interact synergistically, and all will utilize the Biostatistics Core (James Hughes) and Laboratory Core (Walter Stamm). Sheila Lukehart proposes the STI TM CRC Developmental Awards Program.

**Grant:** 2U19AI031494-14  
**Program Director:** FRIEDMAN, HEIDI B.  
**Principal Investigator:** SPINOLA, STANLEY M MD  
**Title:** Midwest STIs Topical Microbicide Cooperative Research  
**Institution:** INDIANA UNIV-PURDUE UNIV AT INDIANAPOLIS, IN  
INDIANAPOLIS  
**Project Period:** 1997/07/01-2009/07/31

**DESCRIPTION** (provided by applicant): This application is the third renewal of the Midwest Sexually Transmitted Infections and Topical Microbicides Cooperative Research Center, a consortium agreement among Indiana University, Northwestern University and the University of Iowa. The overarching theme of the Midwest Center is epidemiology, acquisition, prevention and pathogenesis of STIs in young women. Our goals are to examine: (1) the acquisition of STIs in a cohort of adolescent and young adult women and the developmental factors that are associated with their use and acceptance of topical vaginal microbicides; (2) how HSV-2 and *Neisseria gonorrhoeae* gain access to human cells of the female genital tract, a prerequisite for the development of topical microbicides that block STI entry; (3) mechanisms underlying two sequelae of STIs, cervical cancer and acquisition and transmission of HIV. Of 5 projects, two center on STIs in a cohort of 14 to 24 year old women. Project will (1) focus on developmental factors influencing the acquisition of *N. gonorrhoeae*, *Chlamydia trachomatis*, *Mycoplasma genitalium* and *Trichomonas vaginalis* in adolescents at high risk for STI as they enter young adulthood, (2) assess vaginal microbicide acceptability as a function of relationship status and different ages across adolescence and young adulthood, (3) seek to elucidate signaling pathways involved in internalization of gonococci by primary human cervical cells and will utilize clinical specimens from the adolescent cohort to confirm in vitro findings, (4) define the cell entry pathways used by HSV-2 to infect cells of the female genital tract and of the nervous system in a murine vaginal model of disease, and in human cervical cells. HSV-2 isolates obtained from the clinical cohort will be used to challenge vaccinated mice, (5) examine the role of the HPV E7 protein in cervical cancer by studying the interactions of E7 derived from low risk and high risk viruses with host proteins on the expression of S phase genes and cell cycle exit during differentiation, (6) perform experimental infections with *Haemophilus ducreyi* in human volunteers to address hypotheses about pathogenesis and about interactions between *H. ducreyi* and HIV that facilitate viral acquisition and transmission. The five projects will be supported by Biostatistical, Clinical and Laboratory Cores. The extensive collaboration and cross disciplinary fertilization which exists among the different projects will be reinforced by semiannual scientific meetings and by an external advisory board, which will assess progress of each project and provide constructive criticism and assistance.

**Grant:** 2U19AI031496-14

**Program Director:** FRIEDMAN, HEIDI B.

**Principal Investigator:** SPARLING, PHILIP F MD INTERNAL MED:INTERNAL  
MEDICINE-UNSPEC

**Title:** North Carolina STI/TM Cooperative Research Center

**Institution:** UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC  
HILL

**Project Period:** 1997/07/01-2009/08/31

DESCRIPTION (provided by applicant): This is a competing renewal for a Sexually Transmitted Diseases Topical Microbicide Cooperative Research Center (STD TM CRC). A total of six projects are included, including studies on the STD pathogens *N gonorrhoeae*, *H ducreyi* and *T vaginalis*; epidemiological studies of gonorrhea and syphilis in North Carolina; and clinical and epidemiological studies of the role of HSV and other STDs in facilitation of sexual acquisition of HIV in Malawi. These projects will be supported by an Administrative Core and a Microbiology Core. These studies will unite the scientific areas of basic microbiology and immunology with clinical infectious diseases and epidemiology. There are multiple interactions of the scientific disciplines of molecular genetics, immunology, epidemiology, biostatistics and mathematical modeling. The proposed work is an outgrowth of the current UNC STDCRC but five of the six projects are new. Project 1 studies the interaction between STDs (particularly HSV) and acquisition of HIV in Malawi. Project 2 examines the immunobiology of outer membrane proteins in *H ducreyi* and their potential roles in virulence and vaccines. Project 3 investigates the potential of a gonococcal outer membrane protein PilC as a vaccine candidate. Project 4 examines the potential of several other outer membrane proteins of *Neisseria gonorrhoeae* as a target for topical microbicides or vaccines, using a mouse female genital tract model of infection. Project 5 focuses on developing a molecular typing system for *T vaginalis*; this will enable future epidemiological studies of *Trichomonas* treatment, reinfection, and disease. Project 6 investigates novel spatial mapping and mathematical strategies for disease control and outbreak investigation, using syphilis and gonorrhea in rural NC as the index infections. The overall theme uniting each of these projects is development of novel methods for prevention of STDs.

**DESCRIPTION** (provided by applicant): The San Antonio STI TM CRC proposal represents an integrative, collaborative and innovative multi-disciplinary research effort to investigate and prevent important emerging causes of sexually transmitted diseases. It combines basic and clinical strategies with behavioral and epidemiological analyses and statistical/computing and administrative core support and focuses on underserved South Texas minority women (Mexican- and African-American) and their male partners. The women participants attend a dedicated clinic (designated Project SAFE), which is totally overseen by the San Antonio STI TM CRC and provides clinical evaluation and physical examination of study patients, collects and distributes genital tract and blood samples to specific projects and serves as the collection center for recruitment, interview and intervention activities. The targeted patient population is both understudied and disproportionately affected by STIs. The CRC's goals are to develop and test strategies and tools to understand the biology and infectious potential of specific STIs and prevent and control disease progression through vaccine, diagnostic, microbicide, behavioral and clinical characterizations and interventions. The San Antonio CRC is distinguished by very close collaborations among San Antonio STI TM CRC investigators and additional alliances with other CRC centers and industrial partners. Chlamydia trachomatis, Trichomonas vaginalis, and Mycoplasma genitalium are the emphasized STI agents although correlations with other STIs and urogenital infections are examined through project and core overlap and collaborations. A special strength of the San Antonio CRC is the long-term working relationships that exist among the key investigators, which have been ongoing for many years and are centered in the Departments of Microbiology and Immunology and Obstetrics and Gynecology of The University of Texas Health Science Center at San Antonio. As detailed in the STI TM CRC application the projects are 1: Novel Chlamydia Vaccine Candidates; 2: Mucosal/Oral Antibody Diagnosis for Trichomonas vaginalis; 3: Mycoplasma genitalium Persistence and Diagnosis; 4: Dual STI Prevention Interventions for Minority Couples; and 5: Clinical Aspects of STDs in Dyads. These projects are supported by Statistical/Computing and Administrative Cores.

**Grant:** 1U19AI061972-01  
**Program Director:** FRIEDMAN, HEIDI B.  
**Principal Investigator:** MARTIN, DAVID H MD OTHER AREAS  
**Title:** Gulf South STI/Topical Microbicide Cooperative Res Cent  
**Institution:** LOUISIANA STATE UNIV HSC NEW ORLEANS NEW ORLEANS, LA  
**Project Period:** 2004/09/01-2009/08/31

DESCRIPTION (provided by applicant): The theme of the Gulf South Sexually Transmitted Infections/Topical Microbicide Cooperative Research Center (STI TM CRC) is "Women at high risk for STI's: Focusing research where it is needed the most." There will be four projects and four cores within this CRC. The four projects will focus on the following: 1. Cervical T cell responses to chlamydial infection, 2. The role of Mycoplasma genitalium in infertility in women. 3. The biology of toll-like receptors (TLRs) in genital tract tissues and the potential of TLR agonists as new topical microbicide candidates for use in preventing STI's in high risk women. 4. The role of treatment failure and re-infection in recurrent Trichomonas vaginalis infections following metronidazole treatment. This CRC will have administrative, laboratory, clinical, and biostatistics cores. In addition there are two special aspects of this application. One is a mid-career mentoring program designed to provide senior level guidance to mid-career investigators endeavoring to reach the top of their chosen scientific fields. The other is an important effort to develop modern molecular methods for the study of the vaginal ecosystem that will be included within the Laboratory Core. Our Gulf South STI TM CRC application proposes to create a new CRC in the heart of an area whose population suffers disproportionately from the long term sequelae of STIs. Funding this center will have significant social and public health impact on STIs in this high risk region for many years into the future.



**Grant:** 1U19AI062150-01

**Program Director:** FRIEDMAN, HEIDI B.

**Principal Investigator:** ARNTZEN, CHARLES J PHD BOTANY NEC:BOTANY  
NEC-UNSPEC

**Title:** Plant-Made Microbicides and Mucosal Vaccines for STIs

**Institution:** ARIZONA STATE UNIVERSITY TEMPE, AZ

**Project Period:** 2004/09/01-2009/08/31

DESCRIPTION (provided by applicant): Exclusion of infectious agents from mucosal surfaces of the reproductive tract is a key objective of STI microbicides. The center will analyze mucosal antibodies as mechanisms of exclusion, and complimentary passive and active immunizations to achieve protective antibody concentrations in the female reproductive tract. A unique feature of this center will be the use of green plants as versatile highly cost-effective biomanufacturing systems for microbicides (e.g. antibody-based) and mucosal vaccines. Recognizing that access to STI public health programs will require low-cost and high capacity prevention technologies, this center utilizes the tools of plant biotechnology for biopharmaceutical production. This proposal addresses 4 specific areas of interest. Three scientific project areas are included in the AzBio Center. Project 1 focuses on diversification, characterization and preclinical evaluation of human monoclonals (huMabs) protective against viral STIs. Project 2 focuses on creation and preclinical evaluation of mucosally targeted immunogens to achieve effective vaginal antibody concentrations against viral STIs. Project 3 focuses on the clinical evaluation of passive and active immunization to achieve robust antibody concentrations in the female reproductive tract against viral STIs. A Production Core will provide plant biotechnology services for Mabs and prototype vaccine production to support the three projects. An Administrative Core will oversee and coordinate all of the projects, including defining the regulatory map for clinical evaluation of the technologies, and strategic planning to blend emerging technologies to ensure that new biopharmaceuticals are accessible to the public health community. The outcomes of this study will define the safety and surrogate effectiveness of a layered system of plant-derived public health products for STIs.

**Grant:** 1UC1AI062507-01  
**Program Director:** CASSELS, FREDERICK J.  
**Principal Investigator:** KORNGUTH, STEVEN E PHD BIOCHEMISTRY  
**Title:** Diagnostics for Bacterial/Viral Pathogens Including SARS  
**Institution:** UNIVERSITY OF TEXAS AUSTIN AUSTIN, TX  
**Project Period:** 2004/09/01-2006/08/31

DESCRIPTION (provided by applicant): This research application has as a primary goal the design of a diagnostic platform that will detect genomic sequences of enteric pathogenic bacteria, the Rift Valley fever virus (RVFV), and the SARS-CoV coronavirus. The design involves identification of genomic sequences for the stx and sits pathogenicity islands of enteric bacteria, and of the sequences coding the G and N proteins of RVFV and the S protein of SARS. Very high affinity antibodies and aptamers that bind to these sequences will be prepared and characterized. The genomic probes for the pathogenicity islands and factors and the very high affinity antibodies and aptamers prepared to the protein products of these genes will be coupled to microspheres used in the Luminex assay system. Investigators with expertise in each of these technologies and an industrial partner with expertise in the commercialization of multi-array technology will transition the research to the marketplace. This teaming arrangement provides technologies necessary for the protection of the U.S. from potential biothreats.

**Grant:** 1UC1AI062531-01  
**Program Director:** TAYLOR, KATHERINE A.  
**Principal Investigator:** HENDERSON, IAN PHD  
**Title:** Vitrification-stabilized Multivalent Botulinum Vaccines  
**Institution:** DYNPORT VACCINE COMPANY, LLC FREDERICK, MD  
**Project Period:** 2004/09/01-2007/08/31

DESCRIPTION (provided by applicant): The development of a safe, efficacious vaccine to protect against the seven known naturally occurring serotypes of botulinum neurotoxins would effectively eliminate them as weapons of mass destruction. It is essential that any vaccine include all antigens to protect against all seven serotypes. This is a significant challenge that requires the naturally unstable properties of proteins to be overcome. The aim of this project is to develop ultrastable heptavalent botulinum vaccines. DynPort Vaccine Company, LLC proposes to achieve this by formulating botulinum vaccine antigens in water soluble sugar glasses that entrap proteins, minimizing protein interactions with themselves and their environment, as well as shielding them from the effects of thermal and chemical stresses. Individually formulated antigens will be combined and prepared as monodisperse suspensions in a Perfluorocarbon. Approved by the FDA for use in humans, these inert chemicals enhance stability and provide a suitable delivery vehicle for human immunization, because of its low viscosity, high lubricity and simple respiratory elimination from the body. Their inclusion ensures that the delivery vehicle and vaccine are in the same vial, further enhancing the utility and management of the vaccine. The resulting ultrastable formulations will effectively eliminate the cold chain of vaccine management, constraining vaccine replacement to use rather than stability. We believe that we are uniquely placed to meet the aim of this program. We are funded through the DoD and NIAID to develop multivalent recombinant botulinum vaccine formulations, and the scientific and technical knowledge at DVC will be leveraged to facilitate vaccine formulation design intended for potential future CGMP production. DVC is partnering with Cambridge Biostability Ltd, which has developed and demonstrated this formulation technology for several vaccines. Finally, DVC is also partnering with Battelle Memorial Institute, which has a proven track record in the performance of assays to assess the potency of the botulinum neurotoxin related vaccines and therapeutics.

**Grant:** 1UC1AI062559-01  
**Program Director:** SIZEMORE, CHRISTINE F.  
**Principal Investigator:** NATHAN, CARL F MD INTERNAL  
MED:IMMUNOLOGY  
**Title:** Dihydrolipoamide Acyltransferase:Target for Chemotherapy  
**Institution:** WEILL MEDICAL COLLEGE OF CORNELL UNIV NEW YORK, NY  
**Project Period:** 2004/09/15-2007/08/31

DESCRIPTION (provided by applicant): *M. tuberculosis* (Mtb) is a global health problem and bioterrorism threat but little new chemotherapy against Mtb has emerged in decades. A fresh approach to chemotherapy is to target enzymes the pathogen needs to survive in the metabolic niche it occupies in the host, and/or enzymes that the pathogen needs to resist the stresses that are imposed on it by the host immune response. The metabolism of Mtb in the host depends on oxidation of fatty acids as a carbon source and synthesis of fatty acids as precursors of cell wall lipids. An early stage of fatty acid synthesis is the formation of acetyl-coenzyme A (acetyl-CoA) and other very short-chain acyl CoA's, chiefly from pyruvate dehydrogenase (PDH) and branched chain keto-acid dehydrogenase (BCKADH). A major host defense against Mtb is the imposition of oxidative/nitrosative stress. Remarkably, Mtb appears to rely on one enzyme as a shared component of PDH and BCKADH, and this enzyme also helps to protect the bacillus against oxidative/nitrosative stress: Rv2215 or dihydrolipoamide acyltransferase (DLAT) (formerly "SucB"). Thus, inhibitors of DLAT may cripple Mtb at multiple levels: by interfering with operation of the TCA cycle for generation of ATP and precursors of heme and amino acids; by interfering with synthesis of acetyl CoA, propionyl CoA and acetoacetyl CoA for the glyoxalate shunt and synthesis of fatty acids; and by interfering with antioxidant defense. We have screened a combinatorial chemical library and identified relatively potent and specific inhibitors of Mtb's DLAT. These compounds kill *M. bovis* BCG and Mtb in culture while sparing homologous host enzymes and macrophages. The goal of this application is to use these findings as a rationale, and these inhibitors as a starting point, to develop a compound for testing in mice as a potential therapeutic for tuberculosis with a new mechanism of action.

**Grant:** 1UC1AI062579-01  
**Program Director:** BEANAN, MAUREEN J.  
**Principal Investigator:** BARANY, FRANCIS  
**Title:** Multiplexed Detection of Bioterror Agents  
**Institution:** WEILL MEDICAL COLLEGE OF CORNELL UNIV NEW YORK, NY  
**Project Period:** 2004/09/15-2007/08/31

**DESCRIPTION** (provided by applicant): The current biothreat to our nation requires the ability to rapidly detect and distinguish bioweapon agents from normal pathogens. Existing detection systems have a limited ability to simultaneously screen in a single sample for multiple agents and their antibiotic resistance, toxin, or virulence genes. To meet this need we propose to use ligase detection reaction (LDR) techniques combined with PCR, capillary electrophoresis, and Universal Arrays, which we have already validated in the detection of cancer gene mutations and the diagnosis of genetic diseases. The studies will include the evaluation of Quantum-dots (Q-dots) as a novel detection approach. The above techniques will be used to meet the specific aims of this proposal: Aim 1: To identify multiple blood-borne pathogens simultaneously, in a single assay. Samples will be evaluated for 16 common bacterial pathogens as well as four blood-borne bioterror agents (*B. anthracis*, *Y. pestis*, *F. tularensis*, *B. abortus*). Blood specimens from patients with suspected bacteremia will be evaluated in the microbiology laboratory and tested using the above molecular techniques. Random samples will also be spiked with DNA of bioterror pathogens to simulate infection with these agents. Likewise, HIV RNA will be used as a surrogate for RNA hemorrhagic fever viruses. Aim 2: To evaluate antibiotic resistance, enhanced virulence, or genetic manipulation in a positive blood culture. LDR/PCR will be used to detect the presence of the *vanA*, *vanB*, *mecA*, *tetL*, *tetM*, *gyrA* and *grrA* mutations, additional antibiotic resistance determinants, virulence and toxin genes, which may be present in the common blood-borne pathogens identified in Aim 1. Aim 3: To distinguish Category ABC, engineered, or emergent biothreat agents from common pathogens employing high throughput screening platforms using microbial signature profiles. Standardized protocols using common liquid handling robotic platforms will be established using the techniques validated in Aims 1 and 2. The test will be validated for detecting the above pathogens and further viral agents: Ebola, Marburg, Lassa, Crimean-Congo, Hemorrhagic Fever, Rift Valley Fever, Yellow Fever, SARS Coronavirus, Dengue, and West Nile virus, as well as to distinguish Variola from Vaccinia. In addition, the LDR/PCR virulence gene test from Aim 2 will be expanded to include the major BT toxin and virulence genes. Once verified, our tests will be validated with clinical samples at the CDC.

**Grant:** 1UC1AI062613-01  
**Program Director:** GIOVANNI, MARIA Y.  
**Principal Investigator:** KENNEDY, GUILIA C PHD  
**Title:** Pathogen and Genetic Element Detection Using Microarrays  
**Institution:** AFFYMETRIX, INC. SANTA CLARA, CA  
**Project Period:** 2004/09/30-2007/09/29

DESCRIPTION (provided by applicant): Recent worldwide events have highlighted the need for diagnostic tools to identify bioterrorism agents and pathogens causing human diseases. Current detection methods for those agents do not satisfy the need for high sensitivity, specificity and speed in the detection of those agents. This application proposes to develop and validate a microarray based rapid pathogen detection method (less than 4 hours) that can be used to type multiple pathogens in a single experiment, with high sensitivity and specificity. In addition, this technology will be used to identify genetically manipulated strains, through the detection of antibiotic resistance and toxin genes.

Affymetrix and the Institut Pasteur in France propose to collaborate on the development of this application. The goal of the project is to be able to identify multiple pathogens (bacteria and viruses) simultaneously, but also to identify genetic manipulations in these organisms. Early detection of biological agents and genetic manipulations will allow for earlier initiation of defense mechanisms, evacuations and a fast medical response for the affected areas and people.

Specifically, we propose to: 1. Design a high-density oligonucleotide resequencing array for the identification of 18 bacterial pathogens from the NIAID priority list using their unique 16S rRNA gene sequence, and 25 viruses using organism specific gene sequences; 2. Design a high-density oligonucleotide expression array for the identification of genetically manipulated organisms using known toxin and antibiotic resistant genes; 3. Develop and test a target preparation method that utilizes universal PCR amplification for the selected bacterial pathogens, a multiplex amplification system for viral organisms and a whole genome amplification method to detect genetic elements like toxins and antibiotic resistance genes; 4. Develop a sample preparation and hybridization protocol which, combined, takes less than 4 hours to complete; and 5. Develop and test novel algorithms for the analysis of the pathogen resequencing array to automatically identify and type individual bacteria and viruses in clinical samples and to detect potential genetic elements in these organisms.

**Grant:** 2R01AR042541-10A2  
**Program Director:** TYREE, BERNADETTE  
**Principal Investigator:** HUDSON, ALAN P  
**Title:** Reiter's syndrome mechanism of chlamydial pathogenesis  
**Institution:** WAYNE STATE UNIVERSITY DETROIT, MI  
**Project Period:** 1993/09/30-2009/03/31

DESCRIPTION (provided by applicant): Genital infection with the bacterial pathogen *Chlamydia trachomatis* is associated with development of reactive arthritis (ReA). While it is clear that the process leading to joint disease is partly immunopathogenic in nature, the means by which *C. trachomatis* initiates and maintains that process remain to be elucidated. Data from this group and others have shown that synovial *Chlamydiae* display unusual metabolic and transcriptional characteristics and are arrested at a late stage of the developmental cycle. *Chlamydiae* displaying these and other unusual biologic attributes *in vivo* are designated to be in the persistent state. Accumulating data further indicate that persistent *C. trachomatis* cells interact in an overt but poorly understood manner with their host cells. The key to development of effective therapies to treat *Chlamydia*-associated ReA lies in understanding the biology of chlamydial persistence, and the means by which host and pathogen interact during establishment of that state. In the studies proposed here, we define the genes and gene sets from *C. trachomatis* that are involved directly or indirectly in establishment and maintenance of the persistent state, using a well-characterized *in vitro* model of chlamydial persistence. We also, and coordinately, define the changes in expression for specific, targeted sets of host genes as a function of establishment of persistent chlamydial infection in the *in vitro* model of persistence. The gene sets to be targeted in these analyses will include those from the immune system, the signal transduction system, the energy transduction system, and others. Together, these studies will provide critical new insight not only into chlamydial proteins required for persistence, but also into previously unaddressed interactions between *C. trachomatis* and its primary host cells. Using information gained from these studies, we will determine the molecular genetic basis for differences between patients who progress to chronic disease and those who do not following genital chlamydial infection, and we define the molecular basis for the remittingrelapsing phenotype of patients with chronic *Chlamydia*-induced arthritis, and. These latter studies will give important information relating to host-pathogen interaction during various stages of disease progression. Taken together, the results of the studies proposed here will provide a comprehensive understanding of chlamydial persistence and host-pathogen interaction in ReA and therefore will form the foundation for design and implementation of rational strategies to treat the disease.

**Grant:** 2R01AR043521-11  
**Program Director:** SERRATE-SZTEIN, SUSANA  
**Principal Investigator:** WEIS, JANIS J  
**Title:** Molecular genetics of Lyme arthritis susceptibility  
**Institution:** UNIVERSITY OF UTAH SALT LAKE CITY, UT  
**Project Period:** 1994/09/30-2009/05/31

DESCRIPTION (provided by applicant): Lyme disease is caused by infection with the tick-transmitted spirochete *Borrelia burgdorferi*. Infection in humans can cause inflammatory arthritis in approximately 60% of those not treated at the time of the tick bite, and this can progress to chronic disease in a small percentage of susceptible individuals. The infection-associated arthritis can be studied in mice; with strong evidence suggesting that the severity of *B. burgdorferi* induced arthritis is regulated by the genetics of the host. Using intercross populations of mice developed by crossing inbred strains that develop severe Lyme arthritis (C3H) with those that display milder arthritis (C57BL/6), we have identified six Quantitative Trait Loci (QTL) that regulate arthritis severity. These QTL are highly significant, with LOD scores ranging from 3.5-10.2. Congenic lines have been developed to isolate individual chromosomal intervals associated with QTL by backcrossing seven generations to the reciprocal parent. Several interval specific congenic lines with highly penetrant Lyme arthritis phenotypes have been generated. The goals of this application are to 1) complete the characterization of the C3H x C57BL/6 congenic lines and develop interval specific recombinant lines to narrow the physical region associated with each QTL to that amenable to positional cloning; 2) determine if *Ncf1*, a component of the phagocyte NADPH oxidase, is a candidate for a highly significant QTL mapping to chromosome 5 (Bb2); and 3) determine the mechanism by which reactive oxygen intermediates suppress *B. burgdorferi*-induced arthritis in mice. These studies will provide information on the genetic regulation of Lyme arthritis, with the ultimate goal being the identification of polymorphic genes responsible for differences in severity in mice and humans. Studies with a strong candidate gene, *Ncf1*, have further revealed an unexpected paradigm in inflammatory regulation, suggesting that reactive oxygen intermediates may play a regulatory role in Lyme arthritis.



**Grant:** 1R01AR049812-01A2  
**Program Director:** SERRATE-SZTEIN, SUSANA  
**Principal Investigator:** WASHBURN, LEIGH R  
**Title:** Membrane Proteins and Mycoplasma arthritis  
**Institution:** UNIVERSITY OF SOUTH DAKOTA VERMILLION, SD  
**Project Period:** 2004/08/03-2007/04/30

DESCRIPTION (provided by applicant): Mycoplasma arthritis is a natural pathogen of rats. Although natural infections are now rare, experimental arthritis can be induced by intravenous or intraperitoneal injections. The resulting disease is characterized by rapid onset of acute, inflammatory polyarthritis, followed by a chronic, nonmigratory phase lasting six weeks or longer in some animals. This disease has been used extensively in the past as a model for studying human arthritis, and it remains a convenient and inexpensive system for examining certain inflammatory and immunologic aspects. This study will focus on two M. arthritis surface lipoproteins, MAA1 and MAA2, both of which contribute to adherence to host tissue, are highly immunogenic for infected animals, and induce protective immunity in rats. The central hypothesis is that these proteins are important in pathogenesis of M. arthritis-induced arthritis, possibly by mediating or facilitating adherence to rat tissues. This study will further define their roles in this process. The first aim of this proposal is to prepare genetic constructs to be used in testing this hypothesis. Mutants will be constructed in which the genes encoding both proteins are disrupted by transposon mutagenesis. These mutants will then be subjected to genetic complementation to reintroduce wild-type alleles of the disrupted genes. The second aim is to test these mutants and their genetically complemented counterparts for the ability to attach to rat cells in vitro and to induce arthritis in rats. This study will help to establish the function of these important proteins in the pathogenesis of M. arthritis-induced arthritis. This, in turn, will provide insight into how similar events may occur in inflammatory joint diseases of humans and other animals.

**Grant:** 1R03AR050656-01  
**Program Director:** SERRATE-SZTEIN, SUSANA  
**Principal Investigator:** LI, CHUNHAO PHD  
**Title:** Role of motility in *Borrelia burgdorferi* pathogenesis  
**Institution:** WEST VIRGINIA UNIVERSITY MORGANTOWN, WV  
**Project Period:** 2004/04/19-2007/03/31

DESCRIPTION (provided by applicant): Lyme disease is the most prevalent arthropod borne infection in the United States. The disease, caused by the spirochete *Borrelia burgdorferi*, is a multiple systemic disorder with various clinical manifestations. In the United States, one of the major manifestations of the acute disease is Lyme arthritis. Approximately 60% of untreated patients develop intermittent attacks of monoarticular or oligoarticular arthritis, primarily in large joints such as knees. Previous studies indicate that *B. burgdorferi* is highly invasive. These spirochetes traverse the intercellular matrix, penetrate the vascular endothelial cell lining, and finally invade the joints after being deposited in the skin following a tick bite. However, the mechanisms involved in this invasive process is still unknown. The present proposal focuses on the motility of *B. burgdorferi*, and its role in the disease process. Only recently have the tools for gene targeting been developed for *B. burgdorferi*. In addition, an understanding of its complex motility is at a very early stage. I hypothesize that the flagellar genes *fliG2* and *fliG1* play critical but different roles in *B. burgdorferi* motility. Preliminary results suggest that a *fliG1* null mutant continuously swims but is unable to translate (i.e. show displacement), and a *fliG2* null mutant is completely non-motile. I also hypothesize that *fliG1* functions to coordinate the rotation of the motility organelles, the periplasmic flagella, that allow for directed cell movement. To test these hypotheses, I will characterize these mutants in detail. Green fluorescent protein fusions, and the yeast two hybrid system, will be used to analyze the function of *FliG1* and *FliG2* in depth. The information obtained should yield a better understanding of molecular mechanisms of *B. burgdorferi* motility. Second, I hypothesize that motility is a virulence factor. To test this hypothesis, I will target these two genes in a difficult to manipulate virulent strain, analyze the resultant mutants in depth, and test the virulence of these mutants by the mouse model of Lyme disease. I predict that these two mutants will be less virulent than the parental strain. The results obtained will yield critical information on *B. burgdorferi* motility and its relationship to virulence. These results could lead to new means of disease prevention and treatment.

**Grant:** 1P01AT002620-01  
**Program Director:** WONG, SHAN S  
**Principal Investigator:** PEDEN, DAVID B MD  
**Title:** Translational Research Center for CAM Therapy of Asthma  
**Institution:** UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC  
HILL  
**Project Period:** 2004/09/30-2009/06/30

The objective of this Translational Research Center for CAM therapy of Asthma is to identify antioxidant Complementary and Alternative Medicine therapies for application in asthma. To achieve this objective, we have assembled a team of investigators uniquely qualified for their roles in this project. Project 1 (Phase I Clinical Screening of CAM therapies for asthma, Drs. Peden, PPG PI, Alexis, Bromberg & Patel, UNC) will screen the effect of CAMs initially identified by Drs. Jiang and Ames (gamma tocopherol) or newly identified by Projects 2 & 3 in allergic asthmatic volunteers for Phase I proof of concept studies to determine if CAM therapies protect against endotoxin, O<sub>3</sub>, and allergen induced airway inflammation. Project 2 of the PPG (Preclinical evaluation of CAM therapies for asthma, Drs. Wagner & Harkema, MSU) will evaluate anti-inflammatory CAMs (or CAMs + traditional therapies) identified by Project 3 in allergen sensitized rodents determining if these CAMs, alone and in combination with traditional asthma therapies, blunt exacerbation of airway inflammation due to allergen, endotoxin and ozone (commonly encountered causes of asthma exacerbation). They will also carry out classic animal pathology and pharmacokinetic studies of CAMs. Project 3 of the PPG (Mechanistic Discovery of CAM Therapies for Asthma, Drs. Jiang, Illek and Ames at CHORI), will employ molecular and cellular techniques to examine the actions of tocopherols, ascorbate, polyphenols (genistein and resveratrol) on generation of oxygen radicals by MPO and EPO, mediator production via cyclo-oxygenase and lipoxygenase, and cellular activation via MAP kinases and NF- $\kappa$ B, and impact on epithelial cell physiology. A Biochemistry Core (Dr. Ames, core leader) will assess samples for products of tocopherol oxidation and oxidative burst and a Biomarker and Sample Repository Core (Dr. Patel, core leader) will provide support for cytokine and mediator assessment of samples and tissues for all projects. These studies will be coordinated via quarterly conference calls and semi-annual meetings of the project and core leaders rotated among the study sites. UNC will maintain a server accessible by all investigators to facilitate data sharing and planning of eventual human volunteer studies. Use of antioxidants is an under-investigated area and it is highly likely that these CAM agents will be effective in prevention of asthma exacerbation mediated by inflammatory processes.

**Grant:** 1R01AT002086-01  
**Program Director:** KLEIN, MARGUERITE  
**Principal Investigator:** BARBOSA-CESNIK, CIBELE T MD  
**Title:** Cranberry Juice and Urinary Tract Infections  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2004/01/15-2007/12/31

DESCRIPTION (provided by applicant): Urinary tract infection (UTI) is one of the most commonly acquired bacterial infections in ambulatory and hospitalized populations and E.coli is the most common urinary pathogen, accounting for 90% of UTIs acquired in the community. Antimicrobial treatment and prophylaxis has resulted in increasing resistance to antimicrobials among uropathogenic bacteria both in the United States and worldwide. Several observational studies and a few randomized trials suggest that cranberry juice reduces the incidence of UTI. Reduced incidence of UTI could decrease antibiotic use and ultimately minimize prevalence of antibiotic resistance. Our overall goal is to determine the effect of cranberry on reducing the rate of recurrent UTI and duration of symptoms over antibiotics alone. We propose a randomized clinical trial of 600 college women presenting to the University Health Service with acute urinary tract infection. The study will have 3 arms and patients will be randomly assigned to taking 8 ounces of juice twice a day containing either 27% cranberry juice, 13.5% cranberry juice, or placebo juice. In addition, we will determine whether regularly taking cranberry juice changes the 3 and 6 month prevalence of bladder, rectal, vaginal and periurethral colonization with E coli containing known uropathogenic virulence factors relative to placebo controls. The results of this study will increase our understanding of the cranberry juice effect on reducing the symptoms of acute UTI, and on preventing recurring UTIs. We will be able to understand the dose-response effects on the outcomes of interest, including on side effects and compliance. We will also be able to evaluate if the bacterial population in the vagina, periurethra and stool is different after regularly drinking cranberry juice compared to placebo.

**Grant:** 1R21AT001317-01A1  
**Program Director:** PONTZER, CAROL  
**Principal Investigator:** MAHADY, GAIL B PHD  
**Title:** Botanicals for Chlamydia pneumonia infections-Revision 1  
**Institution:** UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL  
**Project Period:** 2004/02/01-2006/01/31

DESCRIPTION (provided by applicant): Chlamydia pneumonia (CP), an intracellular gram-negative bacterium, is a significant human pathogen linked with 5-10% of cases of pneumonia, bronchitis, asthma and sinusitis. Infection with CP is wide spread with 50-70% of adults worldwide being seropositive. A substantial body of evidence has linked chronic CP infections with the development and progression of atherosclerosis and coronary heart disease (CHD). In 2002 alone over 55 new publications have indicated a strong association between CP and CHD. A variety of antibiotics have been used for the treatment of CP infections, however CP is difficult to eradicate, and requires long-term antibiotic therapy. Thus, antibiotic resistance and treatment failures are common. Therefore new approaches to the prevention and treatment of chronic CP infections are urgently needed. Since many botanical products have been used for centuries for the treatment of CHD and infections, we are proposing to investigate the specific effects of standardized botanical extracts on the pathogenesis of CP and its chronic sequelae. The primary goal of the project is to test standardized botanical extracts and combinations of extracts for the prevention and treatment of CP infections, and determine their impact on atherosclerotic plaque development. To accomplish these goals, the extracts will be tested in vitro to determine activity against CP, as well mechanistic studies. Only the most active extracts will also be tested in an animal model. Over the two-year period 12 botanical extracts will be evaluated in vitro and six in vivo. Botanical selection was based on prioritization using complex data analysis of the Napralert database and other literature sources. Data from our preliminary work demonstrates that the hypothesis and methods for this project are scientifically sound, as a number of extracts used traditionally for the treatment of CHD have already shown activity against CP in vitro. These extracts have since been standardized (based on the active constituents) to enhance the activity, and are ready for animal testing. This proposal is a multidisciplinary collaboration with the long-term objective of developing safe and effective standardized botanicals for the prevention/treatment of CP infections, with overall impact on the development of atherosclerosis and CHD. Data and active standardized extracts developed during this work will be used as the basis for future clinical trials.

**Grant:** 1R21AT001892-01A1  
**Program Director:** KLEIN, MARGUERITE  
**Principal Investigator:** SNYDMAN, DAVID R MD  
**Title:** Probiotic Lactobacillus GG to Eliminate VRE Colonization  
**Institution:** NEW ENGLAND MEDICAL CENTER HOSPITALS BOSTON, MA  
**Project Period:** 2004/09/15-2007/05/31

DESCRIPTION (provided by applicant): Long term carriage of Vancomycin resistant enterococci (VRE) is becoming a major public health problem in hospitalized patients, in patients in nursing homes, and among immunosuppressed populations. Eradication of this organism from colonized patients is difficult and usually unsuccessful. Patients who become infected with this organism are at risk of significant morbidity and mortality. Based on our preliminary data indicating that a probiotic, *Lactobacillus rhamnosus* GG (LGG) broth filtrate is bactericidal to VRE in vitro, we propose to conduct a pilot trial to evaluate whether LGG or placebo is effective in the elimination of VRE among those patients already colonized with VRE. The goals of the study are to determine the extent to which LGG will eliminate VRE from the gastrointestinal tract of colonized individuals compared to placebo, to assess the feasibility of the use of the probiotic LGG to colonize the GI tract of patients who are colonized with VRE, and to assess the ability of LGG to be used safely in this patient population. We will enroll 38 patients who are colonized with VRE into a randomized, double blind, placebo controlled pilot trial. Nineteen patients will receive a daily dose of  $2 \times 10^{10}$  of LGG, and 19 will receive an identical appearing placebo, for 14 days. Stools will be collected twice weekly from enrollment through 21 days, and at 28 and 56 days. Stools will be tested both semi-quantitatively and qualitatively for the presence of VRE, Enterococci, and LGG. For the primary analysis, the proportion of patients colonized with VRE at day 21 post randomization will be compared between patients in the LGG and control groups using Fisher's Exact test with a two-sided alpha of 0.05. With the proposed sample size, this study will have a power of 80% to detect a statistically significant difference between groups if the VRE colonization rate in the control group remains high at 90% while the VRE colonization rate in the LGG treated group is reduced to 45% at 3 weeks post randomization. A data safety monitoring board will evaluate study safety throughout the trial. This pilot study will enable us to establish whether the probiotic LGG can affect the colonization of VRE in the gastrointestinal tract of ambulatory patients colonized with VRE, and will be used to design a larger intervention should there be preliminary evidence of effectiveness.

**Grant:** 2R01CA059021-12  
**Program Director:** DUBOIS, RONALD J.  
**Principal Investigator:** CLARDY, JON  
**Title:** Structural and Functional Studies on Proteins  
**Institution:** HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA  
**Project Period:** 1992/07/10-2007/12/31

DESCRIPTION (provided by applicant): This proposal focused on the structural and functional characterization of enzymes involved in the biological synthesis of unusual natural products. A DNA-based approach to natural products, an approach where biosynthetic gene clusters are captured and heterologously expressed in host organisms has proven to be a powerful method for discovering new biologically active small molecules, new biosynthetic enzymes with unusual activity, and new signaling paradigms for small molecule-based signaling. The work proposed follows up on each of these themes and also proposes colony hybridization-based approaches to identifying potentially useful DNA sequences prior to heterologous expression. A genome wide scan with a synthetic library that amplifies the molecular diversity of the heterologously expressed libraries is also proposed. This scan, if successful, would provide functional annotation for the endogenous function of previously characterized small molecules. Finally, some polypeptides with antibiotic activity will be investigated.

**Grant:** 2R01CA067529-10A1

**Program Director:** DASCHNER, PHILLIP J

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

**Title:** Helicobacter induced hepatitis and tumorigenesis

**Institution:** MASSACHUSETTS INSTITUTE OF CAMBRIDGE, MA  
TECHNOLOGY

**Project Period:** 1995/05/01-2009/06/30

DESCRIPTION (provided by applicant): Since our isolation and naming of *H. hepaticus* and elucidating it causes hepatitis and hepatocellular carcinoma in A/J, AGAFx, B6C3F1 and AXB mice, several additional novel helicobacters have been identified in hepatobiliary tissue of both humans and animals. To continue dissecting the pathogenesis of these novel enterohepatic helicobacters, we have recently completed sequencing the genome of the prototype of these emerging enterohepatic pathogens, *H. hepaticus*. Using this invaluable sequence data, we can study in detail, with the use of isogenic mutants how different virulence genes play a role in chronic inflammation and tumor induction. Furthermore, given *H. hepaticus* recognized tumor promoting ability with hepatocarcinogens and the increasing recognition of helicobacters in diseased liver tissue of humans where hepatitis B and C virus infection is endemic, we want to ascertain whether *H. hepaticus* can synergize with viral proteins to induced hepatocellular carcinomas. Finally, given the ecological niche of these helicobacters in liver is the bile canaliculi, we will explore how helicobacter products or putative virulence genes of *H. hepaticus* affect colonization dynamics and physiological properties of bile in vivo. *Helicobacter hepaticus* induced hepatitis and hepatocellular carcinoma in laboratory mice provides a powerful model system for the study of human liver disease and liver cancer. Like the human conditions, disease in the mouse model is multifactorial, with genetic and environmental factors contributing to pathogenesis. The complexity of the system necessitates parallel investigation into bacterial virulence factors, gene expression changes in liver tissue, adaptive immune changes driven by cytokines, biochemical changes in bile and acute phase response components in plasma, and of course lesion development in the liver. This proposal takes advantage of cutting-edge technology to investigate each aspect of disease pathogenesis in this model. A long-term goal is to integrate information gained in each of these areas to build a complete picture of the molecular and cellular mechanisms involved in hepatitis and hepatocellular carcinoma. In this way, our focus will shift over the next 5 years from individual Specific Aims to synergistic areas of overlap between candidate bacterial virulence factors, bacterial expression profile analysis, host expression profile analysis (at the cell, tissue, and organ level), and proteome analysis (using immunohistochemistry, western analysis, and analytic biochemistry). A more complete understanding of the etiopathogenesis of hepatobiliary disease and hepatocellular carcinoma is likely to result in new strategies for treatment and prevention of these important human diseases.



**Grant:** 2R01CA070329-08  
**Program Director:** DUBOIS, RONALD J.  
**Principal Investigator:** SMITH, AMOS B  
**Title:** Synthesis of Spongistatin Antitumor Agents  
**Institution:** UNIVERSITY OF PENNSYLVANIA PHILADELPHIA, PA  
**Project Period:** 1996/09/01-2008/02/29

DESCRIPTION (provided by applicant): The principal goal of this research program is to develop a practical synthetic approach to the spongipyranes, a family of architecturally unique bisspirolactone macrolides, which possess extraordinary antitumor activities. Building upon the progress of the 01-03 years, we achieved the total syntheses of both (+)-spongistatin 1 and 2 in the 04-07 years; the former proved to be the shortest synthesis of a spongistatin reported to date, with a longest linear sequence of 29 steps (other published sequences are ca. greater than or equal to 32 steps). Equally important, we have developed a highly efficient (22 steps, 4% overall yield), second-generation synthesis of the requisite advanced ABCD aldehyde, capable of producing gram quantities (*vide infra*), which when combined with an equally viable synthesis of the advanced EF Wittig salt, will permit construction of at least one gram of totally synthetic spongistatin 1 for pre-clinical development. The specific aims (A-E) for the next four years are: (A) to complete a highly efficient third-generation synthesis of spongistatin 1 capable of delivering a minimum of one gram, in order to make this important antitumor agent available for pre-clinical evaluation; (B) to provide advanced intermediates for in depth structure-activity studies; (C) to design and synthesize, guided by the structural activity results, less complex analogs possessing significant antitumor activity; and (D) to prepare photo-affinity, fluorescent and radiolabels to define both the site and molecular mechanism of action of these important antitumor agents. Notwithstanding the enormity of this challenge, we are convinced that the synthetic plan described herein will prove superior to isolation as a practical and environmentally sound source of this exciting and immensely important anticancer agent. In the area of new synthetic methods, the Specific Aims for the next four years will include: (E) the development of new bifunctional reagents based on the Brook rearrangement to construct a variety of structurally complex structures via multicomponent one-flask reactions.

**Grant:** 2R01CA081635-07A1  
**Program Director:** DUBOIS, RONALD J.  
**Principal Investigator:** RYCHNOVSKY, SCOTT D  
**Title:** Synthesis of anticancer agents using Prins cyclization  
**Institution:** UNIVERSITY OF CALIFORNIA IRVINE IRVINE, CA  
**Project Period:** 1998/09/04-2008/08/30

DESCRIPTION (provided by applicant): The goals of this proposal are to develop new reactions based on the Prins cyclization and to apply these new reactions to the synthesis of complex natural products. New methods to be developed include the second generation Mukaiyama aldol Prins (MAP) reaction with unactivated alkenes. A new cascade cyclization reaction between enones and enol ethers has been discovered that will be investigated. The mechanism of the Prins cyclization will be studied, and the role of 2-oxonia Cope rearrangements will be investigated. The surprising axial selective in Prins cyclizations promoted by TMSBr cyclization will be studied both mechanistically and as a practical preparative procedure. Syntheses based on this new methodology include routes to calyxin I, a second-generation synthesis of leucascandrolide A, lasonolide A and kendomycin. Both leucascandrolide A and lasonolide A have shown potent cytotoxicity against human cancer cell lines, and the planned syntheses are more concise and efficient than any yet reported for these important compounds. The work described herein will advance the state of synthetic chemistry and provide new tools for organic chemists. These tools will facilitate the synthesis of many new structures that may be important as new pharmaceutical agents.

**Grant:** 2R01CA082312-05A1  
**Program Director:** DASCHNER, PHILLIP J  
**Principal Investigator:** DUBOIS, ANDRE T  
**Title:** BACTERIAL & CHEMICAL CARCINOGENS IN GASTRIC ONCOGENESIS  
**Institution:** HENRY M. JACKSON FDN FOR THE ADV ROCKVILLE, MD  
MIL/MED  
**Project Period:** 2004/09/01-2008/08/31

DESCRIPTION (provided by applicant): Gastric Carcinoma (GC) is the second leading cause of cancer death worldwide. It is believed to result from the co-carcinogenic effects of dietary nitrosamines and *Helicobacter pylori* infection. *H. pylori* persists in the gastric mucosa of >50% of humans worldwide for the life of the host, despite intense immune and inflammatory responses, gastric acidity, peristalsis, and epithelial turnover. During the current cycle of this grant, as planned, we investigated the effect of *H. pylori* infection on the gastric mucosa of the rhesus monkey. We demonstrated that *H. pylori* infection and/or the associated inflammatory response inhibit the expression of a DNA repair gene and thereby may promote cell transformation upon exposure to nitrosamine carcinogens. These results illustrate the complexity of the co-carcinogenic effects of these bacterial and dietary factors. Based on these findings and on the literature, we formulated the following hypotheses: (1) *H. pylori* causes the release of inflammatory mediators and free oxygen radicals that silence DNA repair genes and tumor suppressor genes (TSG), weaken the normal repair mechanisms of epithelial cells and thereby potentiate the effects of chemical carcinogens such as N-ethyl-N'-nitro-N-nitroso-guanidine (ENNG); (2) ENNG in the gastric milieu promotes alterations of the *H. pylori* genome and may increase its virulence; and (3) *H. pylori* is necessary, but not sufficient to cause GC, and removal of *H. pylori* can prevent GC. Our rhesus monkey model is particularly well adapted to test these hypotheses and to fulfill the following specific aims: (1) to characterize the effect of the bacterial carcinogen *H. pylori* and of the chemical carcinogen ENNG on gastric inflammation and DNA damage at the macroscopic, microscopic and molecular level; (2) to explore the effect of ENNG and of the host's responses on the input *H. pylori* genome in placebo- vs. ENNG-treated animals. This portion of the study will determine whether the presence of a carcinogen in the gastric milieu can modify *H. pylori* genome; and (3) to study, in animals that develop GC, the effect of endoscopic mucosal resection alone or combined with *H. pylori* eradication on subsequent GC recurrence. These studies will permit a prospective study of the histological and molecular effects of chemical and bacterial carcinogens during the early and late stages of carcinogenesis.

**Grant:** 2R01CA084374-06  
**Program Director:** HALLOCK, YALI  
**Principal Investigator:** THORSON, JON S PHD  
**Title:** Studies on Calicheamicin Biosynthesis and Resistance  
**Institution:** UNIVERSITY OF WISCONSIN MADISON MADISON, WI  
**Project Period:** 1999/12/01-2009/07/31

DESCRIPTION (provided by applicant): Calicheamicin gamma1 from *Micromonospora echinospora* spp. *calichensis* is the most prominent of the 10- membered enediyne family with respect to its unprecedented molecular architecture, spectacular biological activity and clinical value. As such, calicheamicin is an excellent target for the study of natural product biosynthesis and self-resistance. The objective of the first phase of this study was to i) pursue the biosynthesis of the DNA-delivery component of calicheamicin (the aryltetrasaccharide, comprised of four uniquely functionalized sugars), ii) develop the genetic tools (transformation and gene disruption protocols) to address calicheamicin biosynthesis in *Micromonospora* and iii) investigate the mechanism(s) of calicheamicin self-resistance in *Micromonospora*. With these goals achieved and new tools/information in place, the second phase of this massive project will predominately focus upon expanding this program toward understanding and exploiting the complex biosynthesis of the enediyne core. While continuing our focus upon calicheamicin as a model for 10-membered enediyne biosynthesis, a second complimentary 10-membered enediyne model will be pursued (namely, dynemicin from *Micromonospora chersina*) selected for its unique architecture (an unprecedented fused enediyne-anthracycline), predominate biological activity, anticipated small gene locus size (excellent for production of dynemicin in 'genetically-friendly' heterologous hosts) and the opportunity for comparative genomics of the calicheamicin and dynemicin biosynthetic loci. The fundamental vision of this program remains constant - to present rational strategies from which to build a foundation of knowledge regarding 10-membered enediyne biosynthesis and self-resistance; the consequence of which will continue to provide pioneering discoveries in enzymatic transformation, tools for the rational biosynthetic modification of natural product drug leads, the potential for enediyne overproducing strains and possibly even an enediyne combinatorial biosynthesis program.

**Grant:** 1R01CA099178-01A2  
**Program Director:** WONG, ROSEMARY  
**Principal Investigator:** BORRELLI, MICHAEL J  
**Title:** Heat Activated Gene Therapy Using Radiomimetic CdtB  
**Institution:** WILLIAM BEAUMONT HOSPITAL RESEARCH ROYAL OAK, MI  
INST  
**Project Period:** 2004/09/28-2008/08/31

**DESCRIPTION** (provided by applicant): The central hypothesis is that effective cancer gene therapy can be achieved using a highly toxic transgene that is expressed controllably from a heat-inducible promoter. To test this, cytolethal distending toxin B (CdtB), the cytotoxic component of a tripartite bacterial proteotoxin, was placed into an adenovirus vector under control of a modified human HSP70B heat shock promoter that is extremely silent until heat shocked at 41.0 degrees C or higher. Aim 1 is to optimize using this heat-activatable CdtB gene therapy to establish local tumor control in a rabbit VX2 brain tumor model and to protract animal survival. Aim two is to deliver the adenovirus vectors with tissue permeabilizing agents that increase the interstitial space to promote vector diffusion throughout solid tumors to ascertain if this increases treatment efficacy. It is postulated that using the permeabilizers will increase the fraction of tumor cells that get infected to express CdtB to control tumors more efficiently and permit larger tumors to be treated successfully. Aim three is to explicate the mechanisms of CdtB bystander killing. Treatment efficacy is dependent upon bystander killing of cells adjacent to those infected with and expressing the CdtB transgene. Although CdtB bystander killing has been observed in vitro and in vivo, little is known about it. Experiments will determine if bystander killing is mediated by a freely diffusible extracellular signal or if cell-cell contact and gap junction communication are requisite. Assays will also establish if the bystander cells die by apoptosis or other death mechanisms and will identify the signals that initiate bystander killing. Some tumors, e.g., glioblastoma multiforme, pancreatic tumors, etc. are more refractory to conventional radiotherapy, and chemotherapy, making local tumor control difficult to achieve. Additionally, many tumors recur locally with high frequency and surgery is often impossible and/or radiotherapy options are limited because surrounding normal tissues have accrued their tolerance radiation dose. Consequently, complementary therapies that can improve local control for primary and recurrent cancers are needed. The proposed study will test the potential of heat-activated CdtB gene therapy to help satisfy this need.

**Grant:** 1R01CA100851-01A1

**Program Director:** HALLOCK, YALI

**Principal Investigator:** VALERIOTE, FREDERICK A PHD  
BIOCHEMISTRY:BIOCHEMISTR  
Y-OTHER

**Title:** Discovery of Anticancer Drugs from Cyanobacteria

**Institution:** HENRY FORD HEALTH SYSTEM DETROIT, MI

**Project Period:** 2004/04/01-2009/03/31

DESCRIPTION (provided by applicant): The overall goal of this project is the discovery and development of new anticancer agents with solid tumor selectivity from leads obtained from marine cyanobacteria. The need for new anticancer drugs is significant given the paucity of agents active against the major solid tumors of man. An underlying hypothesis of our screening strategy is that it will generate drugs active against the major solid tumors (such as lung and colon), which are not effectively treated at present. Marine cyanobacteria are abundant as both free-living and symbiotic tropical organisms, and have a correspondingly rich and diverse secondary metabolism. We propose to produce between 1000 and 1500 extracts per year from field collected and cultured tropical marine microalgae, mainly cyanobacteria, with a focus on those of low natural biomass or found in symbiosis with marine invertebrates, such as sponges and tunicates and to characterize "super-producing" marine cyanobacterial strains. Extracts will also be obtained from collections of tuft-forming marine cyanobacteria and planktonic/thin slime forming marine cyanobacteria for culture as well as cultured cyanobacteria isolated from invertebrate hosts under natural product-eliciting conditions. We will use a unique in vitro disk diffusion assay to both identify solid tumor selectivity in the extracts and to direct the isolation of putative anticancer agent. Drug structure will be determined by using and developing innovative NMR pulse sequences and integrating this with MS and other spectroscopic information. If necessary; we will scale-up the culture or recollect selective species to provide sufficient drug to advance to preclinical studies. The first step requires about 20 mg of drug and incorporates information from in vitro concentration-survival clonogenic studies on a solid tumor with pharmacokinetic information (serum and tumor drug levels). The drug is first formulated for intravenous administration and an HPLC assay is developed to monitor serum and tissue levels. The clonogenic/pharmacokinetic information is analyzed to determine whether the more expensive in vivo therapeutic trial should be undertaken. If positive, then an efficacy trial in tumor-bearing mice will be carried out in at least one xenograft model. Therapeutically active drugs will be pursued outside of this application.

**Grant:** 1R01CA107107-01  
**Program Director:** KNOWLTON, JOHN R  
**Principal Investigator:** MARMORSTEIN, RONEN PHD  
**Title:** Structure-Function of Protein Deacetylases  
**Institution:** WISTAR INSTITUTE PHILADELPHIA, PA  
**Project Period:** 2004/03/01-2009/02/28

DESCRIPTION (provided by applicant): Histone deacetylases (HDACs) were first identified through their ability to deacetylate the epsilon amino group of specific lysine residues within the N-terminal tail regions of histones to promote transcriptional repression or gene silencing. Several deacetylase enzymes that have sequence homology to HDACs have more recently been shown to deacetylate non-histone protein targets in vivo such as the p53 tumor suppressor protein for DNA repair regulation and alpha-tubulin for maintenance of cell integrity, suggesting that these proteins have even broader function than transcriptional regulation. The HDAC proteins fall into three classes and employ two different catalytic mechanisms. Class I and II HDACs show considerable sequence homology within the catalytic domain and do not use a cofactor for catalysis. The class III HDACs belong to the Sir2 protein family and show primary sequence and structural divergence with the class I/II HDACs. In addition, the Sir2 proteins employ a novel catalytic mechanism, whereby protein deacetylation is accompanied by NAD<sup>+</sup> hydrolysis generating a novel O-acetyl-ADP-ribose intermediate and nicotinamide. A particularly exciting area of HDAC research relates to their implicated role in human cancer, including the involvement of the human class I HDACs in acute myeloid leukemia and the class III HDACs in the regulation of the p53 tumor suppressor protein. Indeed, HDAC inhibitors are currently in clinical trials as anticancer agents and hydroxamic acid-based HDAC inhibitors, such as SAHA and TSA, have already shown promising activity against several different solid tumors at well-tolerated doses. Despite the important biological role of HDAC proteins and their involvement in human cancer, their mechanism for catalysis, mode of substrate-specific binding, and the biochemical consequence of HDAC deacetylation is poorly understood. This lack of mechanistic information stems from a paucity of structural information on these enzymes. The overall goal of this project is to elucidate the mechanism of HDAC function through a combined structure/function approach on a subset of biologically well-characterized HDAC model proteins. The Specific Aims of the proposal are to (1) Characterize the structure/function of the yeast Sir2 homologue, Hst2; (2) Characterize the structure/function of the bacterial Sir2 homologue, CobB; (3) Determine the crystal structure of archaeal Af1-Sir2 bound to its cognate archaeal chromatin protein substrate, Alba; (4) Determine the structure of the archaeal Af1-Sir2 substrate, Alba; (5) Determine the structure of the class I/II HDACs, human HDAC6 and yeast Hos3. Together, these studies will provide new molecular insights into the mode of catalysis and substrate-specific binding by HDACs, as well as the biochemical consequence of HDAC deacetylation. Moreover, these studies will provide a scaffold for the design of small molecule inhibitors for specific histone deacetylase enzymes that may have applications for the treatment of HDAC-mediated cancers.

**Grant:** 1R01CA109308-01  
**Program Director:** AULT, GRACE S.  
**Principal Investigator:** DUESBERY, NICHOLAS S PHD  
**Title:** MEK singaling in sarcoma growth and vascularization  
**Institution:** VAN ANDEL RESEARCH INSTITUTE GRAND RAPIDS, MI  
**Project Period:** 2004/07/07-2008/06/30

DESCRIPTION (provided by applicant): Many malignant sarcomas such as angiosarcomas are refractory to currently available treatments. However, sarcomas possess unique vascular properties, which indicate they may be more responsive to therapeutic agents that target endothelial function. MEKs have been demonstrated to play an essential role in growth and vascularization of carcinomas. We hypothesize that signaling through multiple MEK pathways is also essential for growth and vascularization of sarcomas. LeTx is a toxin, which targets receptors that are highly expressed in tumor endothelial cells. However, unlike small molecule inhibitors, which target a single biochemical pathway, LeTx inactivates multiple MEK pathways, which are essential for tumor cell proliferation and vascularization. These properties of LeTx indicate that it is uniquely suited to block sarcoma growth and vascularization. The objective of this proposal is to define the role of MEK signaling in growth and vascularization of human sarcoma and determine whether inhibition of multiple MEKs by agents such as LeTx may form the basis of a novel and innovative therapeutic approach in the treatment of human sarcoma. To do this we will define the basic relationship between signal transduction through MEK pathways and growth and vascularization of human sarcoma-derived cell lines using in vitro and in vivo models. Then, we will apply the concepts we will have derived from these models and extend them to the study of a large number and variety of archived clinical sarcoma samples to establish unifying features of prognostic value.



**Grant:** 1R03CA107959-01A1  
**Program Director:** STEELE, VERNON E.  
**Principal Investigator:** WALMSLEY, AMANDA M PHD  
**Title:** Breaking Tolerance to a Tumor associated Antigen  
**Institution:** ARIZONA STATE UNIVERSITY TEMPE, AZ  
**Project Period:** 2004/09/30-2006/08/31

DESCRIPTION (provided by applicant): Breast cancer is the second leading cause of cancer death in women after lung cancer. Despite progress in the treatment of this cancer, survival rates remain poor for patients with metastatic breast cancer. A more effective preventative therapy or treatment for breast cancer is needed. The success of this project will be the first step in our overall goal of developing a subunit vaccine that will enable control of breast cancer in humans. This research activity aims to determine the feasibility of a plant-derived, breast cancer vaccine by determining if tolerance to the tumor associated antigen, mucin-1 (MUC1) can be broken using an orally delivered, plant-derived vaccine. An epitope from MUC1 was chosen for this study since: it has significant correlation to tumor grade; the re-orientation of the MUC1 protein allows access of anti-MUC1 antibodies to tumor sites that are mostly excluded from normal epithelial tissues; it is highly antigenic and it is found in two common forms of breast cancer. An epitope of MUC1 was fused to the B subunit (LTB) of the heat-labile toxin of enterotoxigenic *Escherichia coli* to target the MUC1 epitope to the antigen presenting cells beneath the lining of mucosal surfaces. We have inserted this fusion protein into a plant expression cassette and transformed *Nicotiana benthamiana* and *Lycopersicon esculentum* (tomato) with the resulting construct. Transgenic plant lines were regenerated and their characterization is underway. Characterization of the transgenic plants will entail establishing whether the gene was inserted into the plant genome and establishing correct folding and concentration of the antigens LTB and MUC1 epitope in plant materials. Plant lines displaying high antigen expression, or elite plants, will be processed by freeze-drying, and transferred to the Mayo Clinic, Scottsdale for testing in a mice feed trial. We believe the novel delivery approach of this vaccine has potential to provide a broad immune response more capable of decreasing if not preventing tumor burden.

**Grant:** 1R03CA109917-01  
**Program Director:** STEELE, VERNON E.  
**Principal Investigator:** KAMAT, ASHISH M MBBS  
**Title:** Chemoprevention of murine urinary bladder carcinogenesis  
**Institution:** UNIVERSITY OF TEXAS MD ANDERSON CAN HOUSTON, TX  
CTR  
**Project Period:** 2004/07/01-2006/06/30

**DESCRIPTION** (provided by applicant): The incidence of bladder cancer in the United States is increasing with 57,400 new cases projected for the year 2003. The majority of bladder tumors present as superficial papillary transitional cell carcinomas and recurrence rates approach 88% at 15 years. New approaches, including chemoprevention, are needed to reduce morbidity from this disease. Our study proposes to evaluate the hypothesis that two classes of agents - a cyclooxygenase-2 inhibitor and a quinolone antibiotic - either alone or in combination, have chemopreventive activity against superficial bladder cancer. Both these agents have been widely used with minimal toxicity, making them attractive for chemoprevention studies in humans. The first specific aim will study the in vitro effects of these agents on a panel of human bladder cancer cells with varying invasive and metastatic potential in order to obtain insights regarding activity against different grades of human bladder tumors. The second specific aim will evaluate the agents' chemopreventive efficacy in vivo. For these studies, we will use the transgenic mouse model in which development of papillary tumors in the bladder is driven by the presence of mutant Ha-ras. The tumors in this model mimic the tumors seen in patients who are considered candidates for chemoprevention studies. We will use MRI to image and monitor the tumors within the bladder of the animals since this will provide a rapid method to assess the efficacy of the chemopreventive agents. The third specific aim will determine the effects of the chemopreventive agents on tissue pathology and will address the hypothesis that the agents will prevent or inhibit the growth of tumors by inhibiting cell proliferation, inducing apoptosis, or both. We believe that this application will meet the stated goals of the program announcement to evaluate new chemopreventive agents, test strategies to prevent cancer in persons/animals at increased genetic risk, and to develop innovative animal models to mimic the human cancer process in order to expedite research in cancer prevention.

**Grant:** 1R03CA110822-01  
**Program Director:** STARKS, VAURICE  
**Principal Investigator:** LIU, GEOFFREY MD  
**Title:** H.pylori, genes and esophageal adenocarcinoma  
**Institution:** MASSACHUSETTS GENERAL HOSPITAL BOSTON, MA  
**Project Period:** 2004/07/01-2006/06/30

DESCRIPTION (provided by applicant): Esophageal adenocarcinoma (EA) has the fastest rising incidence of all cancers in the United States. The overall annual incidence has more than tripled over a twenty year period. These statistics imply an urgent problem that needs addressing, and an environmental component to the etiology and pathogenesis of EA. Although gastroesophageal reflux disease, Barrett's esophagus and obesity are known risk factors for EA, more recently, a protective effect for *Helicobacter pylori* (HP) has been hypothesized based on the results of several studies. One objective of this research is to confirm earlier results in a large sample of over 250 histologically-confirmed incident EA cases. Through a secondary analysis of data and specimens from two separate molecular epidemiologic studies, the association between *Helicobacter pylori* and EA risk will be examined via a case-control design, after taking into account important covariates such as gastroesophageal reflux, Barrett's esophagus, and body-mass index. In addition, there may be host factors such as genetic polymorphisms that modify the relationship between HP and EA risk. Genetic polymorphisms involved in three pathways will be evaluated as potential modifiers of this relationship: (a) genes in the inflammatory pathway, such as IL1-beta; (b) genes involved in free radical formation such as MPO and MnSOD; and (c) several candidate DNA repair genes. Both single nucleotide polymorphisms and haplotypes will be evaluated as potential modifiers of the HP-EA risk association. One long-term goal of this small grant project is to generate information that will ultimately lead to a more comprehensive large scale population-based case-control study of EA risks. This application addresses directly priorities set by the NCI, in particular, the recommendations of the Stomach and Esophageal Cancer Progress Report Group (2002), and may have important impact on current medical practices related to the aggressiveness of treating HP infections.

**Grant:** 1R21CA104815-01  
**Program Director:** HOWCROFT, KEVIN  
**Principal Investigator:** GOODYEAR, CARL S PHD  
**Title:** BCR Mediated Apoptotic Death of Leukemia B Cells  
**Institution:** UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA  
**Project Period:** 2004/03/09-2006/02/28

DESCRIPTION (provided by applicant): B-cell chronic lymphocytic leukemia (B-CLL), the most common non-solid tumor in western societies, currently has no effective treatment. Conventional chemotherapeutic drugs are toxic and not well suited to the biology of the disease. In studies that have begun to elucidate the structural requirements of the induction of in vivo targeted death of primary B lymphocytes, we have shown that the bacterial toxin, Staphylococcus aureus protein A (SPA) forms complexes with membrane B-cell receptors (BCR) that induce programmed cell death. The current studies will characterize the properties of the pro-apoptotic BCR complex, and assess whether co-blocking of other immunological survival signals can enhance this potential therapeutic approach. SPECIFIC AIMS: These studies will evaluate basic mechanisms, and consider the overall relevance and efficacy of treatment in an in vivo model of human B-CLL deletion. Aim 1: To define the nature of the pro-apoptotic ligand-BCR complexes on healthy and leukemic Human B cells. Studies will be carried out to reveal the SpA-BCR complexes formed on healthy and CLL B-cells. Using flow cytometry and de-convoluted con-focal microscopy, we will characterize the pro-apoptotic complexes, and specific constituents required to cause activation and signaling of these B-cells in vitro. Aim 2: To evaluate the sensitivity of B-CLL cells to BCR-induced deletion in an in vivo RAG -/- transfer model. We will investigate the deletion of transferred healthy and CLL B-cells in RAG-1 -/- mice and evaluate whether the specific B-CLL subset can be deleted with SpA (VH3-specific) and also investigate the mechanism(s) associated with B-cell deletion. Aim 3: To evaluate the enhancement of BCR induced apoptosis in B cells by simultaneously blocking alternative immunological survival signals. To further define how BCR targeting can be utilized as a therapeutic reagent, different pathways of activation-induced cell death will be considered and how they can be enhanced by the addition of various inhibitors for immunological survival signals i.e., TACI-Fc or anti- BAFF antibodies.

**Grant:** 1R21CA110494-01  
**Program Director:** WU, ROY S  
**Principal Investigator:** ROTH, MICHAEL D MD  
**Title:** Adjuvant immunotherapy for non-small cell lung cancer  
**Institution:** UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA  
**Project Period:** 2004/08/16-2006/07/31

DESCRIPTION (provided by applicant): Patients with clinical stage IB, IIA/B and IIIA non-small cell lung cancer (NSCLC) have poor 5-year survival rates that range from 15-40%. Preliminary results from an international cooperative study suggest that survival might be improved (approximately 5%) by treating patients with post-operative chemotherapy and/or radiation, although a high percentage of patients (23%) experienced serious Grade 4 toxicity. Adjuvant therapies that are more tumor-specific and less toxic are needed. We propose that dendritic cells (DC) loaded ex vivo with antigens from the patient's own irradiated autologous tumor, and matured with a combination of interferon-gamma (IFN-gamma) and inactivated/formaldehyde-fixed (BCG), can be used to safely induce a state of anti-tumor immunity and prolong tumor-free survival in the adjuvant setting. A Phase I clinical trial will be carried out to evaluate the safety and tolerability of administering this form of DC vaccine to postoperative patients with selected clinical stage IB, IIA/B or IIIA NSCLC. Tumor cells produce immunosuppressive factors that can suppress host immunity and enhance tumor survival. Resection of the primary tumor reduces these factors and temporarily restores immune responsiveness. Tumor resection also reduces tumor burden, reducing the risk that tumor variants will escape immune detection. As such, treating patients in the postoperative period provides an optimal window for employing anti-tumor vaccines. DC precursors will be harvested by leukapheresis and differentiated ex vivo with GM-CSF and IL-4. Autologous tumor will be harvested during the patient's primary tumor resection and purified using a novel immunodepletion protocol. Using the patient's own tumor, DC will be loaded with a full repertoire of tumor antigens capable of inducing both CD4 and CD8 T cell responses. After antigen loading, DC will be treated with BCG and IFN-gamma to enhance IL-12 production, co-stimulatory activity and capacity for T cell activation. The DC vaccine will then be administered as a monthly intradermal injection for three consecutive months following recovery from surgery. A total of 12-15 patients will be treated in two cohorts, one receiving  $2 \times 10^6$  DC per immunization and the second receiving  $6 \times 10^6$  DC per immunization. In addition to standard toxicity, tolerability and clinical outcomes, changes in serum cytokines (IL-10, VEGF, TGF-beta) and cell subsets (DC1, DC2, T suppressor) will be used to monitor changes in tumor-related immunosuppression; skin-test reactions to PPD, BCG-specific T cell proliferation and intracellular cytokine responses to DC loaded with BCG will be used to monitor responses to the vaccine; and T cell proliferation and intracellular cytokine responses to tumor-loaded DC will be used to monitor the development of tumor-specific immunity. Patients will be followed for a total of 1 year. Evidence that the vaccine is safe, documentation of a feasible manufacturing process, and evidence of vaccine-specific immune responses will provide the information required to support subsequent clinical trials focused on clinical outcomes and survival.

**Grant:** 2R37CA044848-18  
**Program Director:** HALLOCK, YALI  
**Principal Investigator:** FENICAL, WILLIAM PHD CHEMISTRY:ORGANIC  
SYNTHESIS  
**Title:** Antitumor-Antibiotics from Marine Microorganisms  
**Institution:** UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA  
**Project Period:** 1987/04/15-2009/02/28

DESCRIPTION (provided by applicant): This is a competitive renewal application to continue to explore marine microorganisms as a new source for antitumor-antibiotics. The proposed project would continue as a collaboration between chemists and microbiologists at the Scripps Institution of Oceanography in La Jolla, CA. and cancer biologists at the Bristol-Myers Squibb Pharmaceutical Research Institute in Princeton, NJ. Studies proposed for the renewal period include the following: The isolation, cultivation and biological evaluation of marine microorganisms from diverse habitats. The focus of the renewal application will be on 7 new groups of obligate marine actinomycetes (Salinospora, Marinomyces, and the MAR 3-7 groups) discovered during the past 24 months. Overall, to continue to isolate and describe new molecules, which show novel mechanisms of action in cancer-relevant, mechanism-based screens show in vitro tissue type selectivity in the NCI 60 cell line and BMS ODCA panels, and to subsequently investigate their in vivo efficacies. To focus a screening effort on the new cancer relevant targets IGF-1 Growth Factor, DNA Methyl Transferase-1 (DNMT-1), Coactivator Associated Arginine Methyltransferase (CARM-1) and Ceramidase, and on yeast selective DNA repair and cell checkpoint targets. These goals will also be supplemented at SIO with several assays, which focus on infectious diseases (screening against drug resistant pathogens and for inhibitors of two selected bacterial efflux pumps). To continue to advance previous discoveries (Salinosporamide A, Halimide, Sargassamide A and several other molecules) for in vivo evaluation in the BMS P388 and L1210 leukemias and the murine solid tumor models M109, Mam16/C and M50766.

**Grant:** 2R01DA013371-04A2  
**Program Director:** SCHNUR, PAUL  
**Principal Investigator:** LYSLE, DONALD T PHD OTHER AREAS  
**Title:** Behavioral Factors in Heroin's Effect on Nitric Oxide  
**Institution:** UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC  
HILL  
**Project Period:** 2000/09/30-2009/07/31

**DESCRIPTION** (provided by applicant): There is a high incidence of infectious disease in heroin-dependent individuals. In spite of the major health issues surrounding heroin use, few studies have examined the impact of heroin on immune status. A wealth of recent data has revealed that nitric oxide plays a pivotal role in determining the outcome of exposure to certain pathogens. Our laboratory has provided the first evidence that heroin administration produces alterations of the expression of inducible nitric oxide. Specific Aim I tests the hypothesis that heroin induces alterations of nitric oxide production in models of Gram-positive, Gram-negative infection, and polymicrobial infection. The proposed studies investigate the effects of heroin on nitric oxide production in models of both Gram-negative and Gram-positive bacterial infection. These studies investigate the dose-dependent effects of heroin on the production of nitric oxide following injection of immunogenic bacterial components: lipopolysaccharide (Gram-negative), lipoteichoic acid alone (Gram-positive) and in combination with peptidoglycan. Subsequent studies will examine the effects of heroin on nitric oxide expression using live bacterial challenges with monoinfections of representative sepsis-capable strains of Gram-negative bacteroides and Gram-positive Group B streptococci, and the ceca-ligation and puncture model (CLP), a polymicrobial infection challenge. Specific Aim II tests the conditioned effects of heroin-induced alterations of nitric oxide expression in models of Gram-positive and Gram-negative infection, and polymicrobial infection. There is growing evidence that Pavlovian conditioning processes can modulate immune responses. Our laboratory has provided the first data showing that environmental stimuli paired with heroin-induce alterations of nitric oxide expression. The proposed studies extend this important discovery to models of both Gram-negative and Gram-positive bacterial infection, and the mixed infection CLP model, including assessments of acquisition and extinction of conditioned responses. Specific Aim III tests the hypothesis that the central dopaminergic systems are involved in the regulation of heroin-induced alterations of nitric oxide expression in models of infection. Our laboratory has provided new data showing that dopamine is involved in the conditioned and unconditioned effects of opioids on immune responses. The proposed investigations examine the role of central dopamine in unconditioned and conditioned heroin-induced alterations of nitric oxide expression. The proposed experiments will determine the effect of 7-OH-DPAT and the D1-selective antagonist, SCH23390, on heroin-induced alterations of nitric oxide in both the component and live models of infection. Collectively, the characterization of heroin-induced modulation of inducible nitric oxide in bacterial injections models provides a greater understanding of how opioids impact the immune system and health.

**Grant:** 1R21DA018816-01  
**Program Director:** HILLERY, PAUL  
**Principal Investigator:** WELLS, GREGG B MD  
**Title:** Bacterial Proteins in the Nicotinic/GABA Receptor Family  
**Institution:** TEXAS A&M UNIVERSITY HEALTH SCIENCE COLLEGE STATION, TX  
CTR  
**Project Period:** 2004/09/30-2006/08/31

**DESCRIPTION** (provided by applicant): Nicotinic acetylcholine receptors and GABAA receptors are members of the nicotinoid family of ligand-gated ion channel receptors of the central nervous system. They play major roles in addiction to nicotine, cocaine, alcohol, and barbiturates. Better prevention or treatment of these addictions will have major impact on human health. High-resolution structures of these receptors are needed as a foundation on for understanding how these receptors work, how they affect addiction, and how receptor-specific drugs can be designed for targeted therapeutics for addiction and also for Alzheimer's and Parkinson's diseases, epilepsy, and mental illnesses. The acetylcholine binding protein is limited as a structural model of nicotinoid receptors, because it is not an ion channel. Structures of functional nicotinoid receptors, however, have been elusive. A major obstacle has been the difficulty of obtaining enough protein, either for X-ray crystallography or with isotopic labeling for NMR. Although no bacterial nicotinoid receptors have yet been identified, such bacterial receptors could, in principle, solve this problem. Similar to the impact that bacterial potassium and chloride ion channels have on understanding those families of channels, bacterial nicotinoid receptors promise to significantly advance the understanding of the structure and function of nicotinoid receptors. We have identified several bacterial proteins whose primary amino acid sequences are similar to the extracellular domain and transmembrane domains of nicotinic acetylcholine, ionotropic GABA, and glycine receptors. The long-range goal of this project is to develop an atomic-level structural interpretation of how nicotinoid receptors contribute to addiction. The objective of this application is to determine whether bacterial proteins belong to the nicotinoid receptor family. The central hypothesis is that bacterial proteins with primary amino acid sequences that are similar to eukaryotic nicotinoid subunits are members of the nicotinoid receptor family. These specific aims test this hypothesis: (1) determine whether bacterial proteins with substantial primary sequence homology to subunits in nicotinoid family assemble into oligomers; (2) determine whether these proteins are transported to the cell surface; and (3) determine electrophysiological properties of these proteins. Identifying the first bacterial nicotinoid receptors will provide promising candidates for structural study of this important family of receptors.



**Grant:** 2R01DC000263-17  
**Program Director:** WATSON, BRACIE  
**Principal Investigator:** CHOLE, RICHARD A  
**Title:** Cellular and Molecular Biology of Cholesteatoma  
**Institution:** WASHINGTON UNIVERSITY ST LOUIS, MO  
**Project Period:** 1985/09/16-2008/11/30

DESCRIPTION (provided by applicant): Cholesteatomas of the middle ear and mastoid develop as a complication of otitis media. These epidermal structures often become infected with a mixture of aerobic and anaerobic bacteria, the most common of which is *Ps. aeruginosa*. Bacteria form biofilms within cholesteatomas resulting in chronic infection. These infected cholesteatomas are aggressive and cause increased osteolysis. We have identified strains of otopathogenic *Ps. aeruginosa* (OPPA) that have increased adherence to keratinocytes and increased biofilm production. We will further characterize these isolates by examining the expression of quorum sensing genes and alginate gene expression and production. We propose to study the pathogenesis and virulence of these chronic infections. With regard to pathogenesis, we will study the adherence of these organisms to keratinocytes using randomly directed mini-Tn5 transposon mutagenesis of adherent OPPA isolates. The mutagenesis screen will be enriched for non-adherent mutant bacteria and will subsequently be sequenced to identify novel adhesin genes. With regard to virulence, preliminary studies show that our OPPA strains produce osteoclastogenesis by both an LPS-dependent and LPS-independent mechanism. LPS-dependent studies will be done with LPS sensitive murine osteoclast precursors. Expression of signals associated with osteoclastogenesis (e.g. RANKL, TNFa) will be determined to understand the mechanism of *Ps. aeruginosa* LPS mediated osteoclastogenesis. To study LPS-independent osteoclastogenesis, we will use LPS-insensitive osteoclast precursors derived from mice deficient in toll-like receptor 4 to determine which portions of the osteoclast development pathway are induced.

**Grant:** 2R01DC002148-12A2  
**Program Director:** WATSON, BRACIE  
**Principal Investigator:** EHRLICH, GARTH D  
**Title:** Molecular Analysis of Pathogens in Otitis Media by PCR  
**Institution:** ALLEGHENY-SINGER RESEARCH INSTITUTE PITTSBURGH, PA  
**Project Period:** 1993/12/01-2009/03/31

DESCRIPTION (provided by applicant): Otitis media (OM) is the most common reason that an ill child visits a health care provider or undergoes a general anesthetic. OM is also the most common reason that a child receives an oral antibiotic and the over-treatment of patients with OM has been suspected of contributing to the development of antimicrobial-resistant organisms. OM disproportionately affects socio-economically disadvantaged children, Native American children and is a factor that inhibits women from full participation in the workforce. The major focus of our laboratory for the past decade has been elucidating the path physiology of chronic OM with the ultimate goal of developing more effective treatments. During this time, we have shown that chronic OM is not a purely inflammatory process, but rather is a bacterial biofilm illness. The recognition that OM is a biofilm disease then led to a novel hypothesis: The Distributed Genome Hypothesis. This hypothesis states that there is a supra-genome for pathogenic bacteria and that each individual bacterium possesses only a subset of genes from the supra-genome. The supra-genome is a reservoir for panoply of contingency genes that collectively provides a significant survival benefit for the population-at-large. In this continuing application we specifically test the Distributed Genome Hypothesis in *Haemophilus influenza* (HI) with four Specific Aims: 1) Perform comparative genomic studies among clinical isolates of HI To characterize the extent of genomic plasticity; 2) Determine the extent of HI inter-isolate recombination during the infectious process; 3) Prepare transformation knockouts of HI and compare their survival time in vivo with wild-type congener strains; 4) Phenotypic characterization of HI biofilms. These Specific Aims will be accomplished by experiments using state-of-the-art high throughput genomic, molecular biology, imaging and modeling techniques, as well as investigations in children. Preliminary data generated from a micro array library composed of 10 clinical isolates of *H. influenza* demonstrated that *H. influenza* does indeed have a supra-genome that is twice the Size of a single bacterium. These findings shed light on many aspects of OM, including disease persistence in the face of antibiotic treatment, and provide an explanation for the success of adenoidectomy in the management of OM.

**Grant:** 2R01DC002873-10  
**Program Director:** WATSON, BRACIE  
**Principal Investigator:** ST GEME, JOSEPH W MD  
**Title:** Biology of the HMW1 and HMW2 Adhesins of H. Influenzae  
**Institution:** WASHINGTON UNIVERSITY ST LOUIS, MO  
**Project Period:** 1995/06/01-2009/06/30

DESCRIPTION (provided by applicant): Nontypable *Haemophilus influenzae* is a common cause of localized respiratory tract disease, especially otitis media, sinusitis, pneumonia, and bronchitis. Following each episode of acute otitis media, fluid remains in the middle ear for weeks to months and is associated with significant hearing deficit, which in turn can impair language acquisition, speech development, and school performance. The initial step in the pathogenesis of disease due to nontypable *H. influenzae* involves colonization of the upper respiratory epithelium. We have demonstrated that two related high-molecular-weight proteins called HMW1 and HMW2 promote attachment to human epithelium, an essential step in the process of colonization. Of note, HMW1- and HMW2-like proteins are present in 75-80% of all nontypable *H. influenzae* strains. In recent work, we have established that the HMW1 and HMW2 proteins are variant members of the autotransporter family and are glycosylated. Based on studies with HMW1, glycosylation requires a protein called HMW1C and a phosphoglucomutase involved in LOS biosynthesis. In addition, we have discovered that expression of HMW1 and HMW2 is phase variable, enabling the organism to adapt to diverse environments and evade the host immune response. In the present proposal we plan to elucidate the molecular details of HMW1 and HMW2 glycosylation. In particular, we will define the chemical structure of the carbohydrate modifying HMW1 and HMW2, the biosynthetic pathway involved in glycosylation of HMW 1 and HMW2, and the relationship between glycosylation and immunogenicity. In additional experiments, we will elucidate the mechanism by which expression of HMW1 and HMW2 is regulated, focusing in particular on the function of 7-base pair tandem repeats that lie within the promoters of the HMW1 and HMW2 structural genes and undergo spontaneous variation in number. We will also examine the role of a conserved 19-base pair sequence that is upstream of the repeats in the promoters of the HMW1 and HMW2 structural genes and upstream of the *hmwB* and *hmwC* genes. Finally, using microarray technology, we will identify *H. influenzae* genes that are activated by HMW1/HMW2-mediated adherence, then assess the effect of these genes in the chinchilla otitis media model. From a practical perspective, the results of these studies may be directly relevant to the development of novel antimicrobials and a licensed vaccine effective in the treatment and prevention of nontypable *H. influenzae* disease. More generally, they may provide fundamental insights into host-microbial relationships, protein secretion, protein glycosylation, and gene regulation.

**Grant:** 2R01DC003915-06  
**Program Director:** WATSON, BRACIE  
**Principal Investigator:** BAKALETZ, LAUREN O PHD  
**Title:** Determinants of H. influenzae Virulence in Otitis Media  
**Institution:** CHILDREN'S RESEARCH INSTITUTE COLUMBUS, OH  
**Project Period:** 1999/09/30-2009/08/31

DESCRIPTION (provided by applicant): Middle ear infection or otitis media (OM) is a highly prevalent pediatric disease worldwide. There were nearly twenty-five million physician's office visits made for OM in 1990, and available evidence suggests that the incidence is increasing. While only very rarely associated with mortality, the morbidity associated with OM is significant. The socioeconomic impact of OM is also great. Direct and indirect costs of diagnosing and managing OM exceed \$5 billion annually in the U.S. alone. Clearly, there is a tremendous need to develop more effective and accepted approaches to the management and preferably, the prevention of OM. Vaccine development holds the greatest promise and would be the most cost-effective method to accomplish this goal. However, progress in terms of vaccine development for nontypeable *Haemophilus influenzae* (NTHI), the Gram-negative pathogen that both predominates in chronic otitis media with effusion or OME, as well as being a significant etiologic agent of acute OM, continues to be hampered by our incomplete understanding of the pathogenesis and immunobiology of OM, a polymicrobial disease caused by one or more of the three predominant bacterial pathogens, whose ability to invade the tympanum is facilitated by virtually any upper respiratory tract (URT) virus. During the past 4 years, we have: sequenced and will soon complete the annotation of the genome of an OM isolate of nontypeable *Haemophilus influenzae* (NTHI); developed and used DNA plasmid based microarrays to conduct strain comparison studies for two clinical OM isolates; developed a promoter trap system in NTHI with which we have monitored gene expression in vivo, during experimental OM; and have developed a signature tag mutagenesis system in NTHI that provided us with a complementary system to identify genes that are essential for colonization and induction of OM. Not only are all these tremendous resources available to us to capitalize upon as we extend our studies of the pathogenesis of NTHI-induced OM, but they have also already resulted in the identification of multiple potential new virulence determinants for NTHI. We propose experiments for the next funding period designed to continue to enhance our understanding of both NTHI pathogenesis in OM at the molecular level as well as further our investigation of a focused group of novel virulence determinants. We will first rigorously assess the feasibility of using lux-expressing NTHI strain 86-028NP to provide a non-invasive, whole animal imaging system to be applied to, and significantly advance, our studies of both pathogenesis and vaccine-mediated prevention of OM. Secondly, we will use a chinchilla super infection model to assess the protective efficacy of one well-developed candidate antigen and one novel and highly promising, but less developed candidate, for ability to prevent ascending OM after intranasal immunization. We will also continue to identify and characterize putative virulence determinants and assess their potential as vaccine or therapeutic targets via a variety of methodologies, including gene expression profiling by microarray analysis. Overall, our studies will lead to an improved understanding of OM caused by NTHI and lead to new strategies to prevent OM.

**Grant:** 1R01DC006917-01  
**Program Director:** WATSON, BRACIE  
**Principal Investigator:** ZHANG, JING-REN PHD  
**Title:** Otitis media-associated pneumococcal genes  
**Institution:** ALBANY MEDICAL COLLEGE OF UNION UNIV ALBANY, NY  
**Project Period:** 2004/07/01-2009/06/30

DESCRIPTION (provided by applicant): *Streptococcus pneumoniae* (the pneumococcus) is the most common cause of acute otitis media (OM) in children. The pathogenesis of pneumococcal infection in the middle ear depends on bacterial adherence to mucosal epithelial cells. Attachment to mucosal surfaces not only facilitates pneumococcal colonization in the middle ear, but also triggers inflammatory responses and induces mucus secretion. Profound inflammation and excessive secretion are the hallmarks of pneumococcal OM. In contrast, the mechanisms underlying pneumococcal adherence to the mucosal surface of the middle ear are poorly understood. Our long-term objectives are to elucidate the molecular mechanisms of pneumococcal adherence, and to evaluate the potential of interfering with pneumococcal adherence as a strategy to prevent OM and other pneumococcal infections. We have already isolated 14 mutants of *S. pneumoniae* that are substantially reduced in their ability to bind to human middle ear epithelial cells. One of these mutants carries an insertional mutation in an open reading frame (designated MplA) that is homologous to the M protein of *Streptococcus pyogenes*. We thus hypothesize that surface proteins of *S. pneumoniae* promote pneumococcal adherence to middle ear epithelium. This proposal will test this hypothesis by systematically identifying and characterizing pneumococcal proteins that are associated with adherence of *S. pneumoniae* to human middle ear epithelial cells. Our Specific Aims are to: 1) Characterize the mechanism of MplA-mediated adherence. We will determine (i) whether the MplA protein is expressed at the surface of *S. pneumoniae*, (ii) which domain of MplA mediates binding to host cells, and (iii) whether the *mlpA* gene and its protein product are conserved in pneumococcal strains isolated from OM patients. 2) Identify the genes encoding new *S. pneumoniae* adhesins. We will accomplish this aim by (i) characterizing the 13-uncharacterdzed adherence-deficient mutants and (ii) screening a library of signature-tagged pneumococcal mutants that were recently established in our laboratory. 3) Determine the ability of adhesins to induce protective immunity against *S. pneumoniae* infection in the middle ear. MplA and other adhesin proteins identified in Aim #2 will be used to immunize the OM rat model. Protective activity will be assessed by (i) bacterial burden and (ii) inflammation in the middle ear following challenge with virulent pneumococci. The resulting information will not only enhance our understanding of OM pathogenesis, but may also lead to improved prevention of OM cause by *S. pneumoniae*.

**Grant:** 1R03DC006585-01  
**Program Director:** WATSON, BRACIE  
**Principal Investigator:** MCCREA, KIRK W PHD  
**Title:** Segregation of H. influenzae LPS genes in otitis media  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2004/01/21-2006/12/31

DESCRIPTION (provided by applicant): Non-typable (NT) H. influenzae is both a human commensal and a major cause of ear infections. The lipooligosaccharide (LOS) of H. influenzae is a well-documented bacterial component contributing to colonization and diseases. Recently, a study utilizing subtractive hybridization identified a LOS biosynthesis gene (lic2B) as being strongly associated with H. influenzae isolated from the middle ear of children with otitis media and compared to isolates obtained from the nasopharynx of healthy children in daycare. These results suggest that LOS genes may be differentially segregated between NT H. influenzae middle ear and throat isolates. More than sixty genes have been proposed to contribute to the generation of LOS. Many of these synthesize highly variable structures that facilitate survival of H. influenzae in different host environments. Given the complexity of LOS, it is likely that numerous LOS gene differences exist between middle ear and throat isolates. We hypothesize that specific LOS "genotypes" are associated with middle ear isolates. AIM I. Determine the distribution of LOS genes among NT H. influenzae middle ear and throat isolates. AIM II. Identify LOS gene variations and determine their distributions among middle ear and throat isolates.

**Grant:** 2R01DE008007-18A1  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** BRADY, L JEANNINE BA  
**Title:** MEMBRANES OF THE DENTAL PATHOGEN STREPTOCOCCUS MUTANS  
**Institution:** UNIVERSITY OF FLORIDA GAINESVILLE, FL  
**Project Period:** 1986/03/01-2007/12/31

DESCRIPTION (provided by applicant): During the previous funding cycle a 5-gene operon named sat, for secretion and acid tolerance, was identified in *Streptococcus mutans*. This operon, located immediately downstream from the opuA osmotolerance operon, contains ffh that encodes Ffh, a key component of the bacterial signal recognition particle (SRP). *S. mutans* ffh mutants displayed acid intolerance and an acid inducible promoter was identified that allowed coordinate expression of the entire sat locus as a single mRNA transcript. Mutants displayed markedly reduced levels of H<sup>+</sup>/ATPase in isolated membranes leading to the belief that an acid tolerance response involves cotranslational translocation of membrane proteins by the SRP. In the present proposal, we will study further regulation of the sat locus, and its regulatory relationship to opuA, by means of Northern blot and real time RT-PCR analyses and investigate the role of YlxM, encoded by y/xM immediately upstream of ffh, as a putative regulatory protein. We will confirm the presence of the SRP in *S. mutans* and determine its similarity to SRPs described from other prokaryotes by searching for the known complexed components, Ffh and the small cytoplasmic (4.5S) RNA, along with the membrane receptor, FtsY, in complexes immunoprecipitated from strain NG8 cellular extracts. We will employ the ffh mutant, MK4, and the wild-type strain NG8 to identify membrane proteins that require the SRP for translocation. Cells grown under steady-state (chemostat) conditions at pH 7 or 5 will serve as the source of cell fractions. Two-D gel electrophoresis, yeast two-hybrid, Ffh-GST affinity chromatography and affinity chromatography will be employed. Major physiologic parameters, including glycolysis and pH homeostasis, and genetic competence will be studied in this mutant which has been shown to have major alterations in these characteristics. Also during the previous funding cycle, the necessity of the central proline-rich repeat domain for surface expression of adhesin P1 (I/II) in *S. mutans*, but not secretion in *E. coli*, was described. The role of gene products encoded within the clp, dnaK and groE operons in the intra-cellular turnover and chaperone-mediated surface expression of P1 will be assessed and intra-molecular interactions of P1 domains necessary for stability and translocation will be evaluated. By complementation of *E. coli* Sec-pathway mutants with an NG8 gene library, *S. mutans* homologues/analogues of Sec components will be identified. Immunoprecipitation of P1 and associated cellular proteins with anti-P1 antibodies will be used to identify chaperones or modifying factors involved in the export of this key adhesin. The project described in this proposal continues studies of membrane biogenesis and translocation of a major surface-localized protein of *S. mutans*.

**Grant:** 2R01DE009838-10A1  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** SCANNAPIECO, FRANK A DMD  
**Title:** Amylase Binding Streptococci Dental Plaque and Caries  
**Institution:** STATE UNIVERSITY OF NEW YORK AT AMHERST, NY  
BUFFALO  
**Project Period:** 1992/09/30-2008/12/31

**DESCRIPTION:** Dental plaque is the oral biofilm responsible for the etiology of Dental caries and periodontal disease. A number of salivary proteins have been shown to interact with bacteria in plaque, and such interactions likely play critical roles in plaque formation. One such interaction is that between amylase, the most abundant enzyme in saliva, and the amylase-binding streptococci (ABS), which are numerous in plaque. Based on our recent findings generated during the previous funding period, this once-amended competitive renewal application seeks to continue our studies of the biochemical, physiological, cariological and ecological consequences of amylase-binding to ABS. We found that while amylase binding-deficient mutants adhere less well to amylase-coated surfaces and demonstrate defective biofilm formation in vitro, they colonize rat teeth better than wild type strains, out-compete their parental strains in rats fed sucrose/starch diet. These surprising findings led to the realization that AbpA inhibits sucrose-dependent colonization determinants such as, but perhaps not limited to, GtfG of *S. gordonii*. Thus, our Aims for the next funding period are to: 1) investigate potential interactions of genes and proteins involved in amylase binding (abpA and abpB) and genes involved in glucan synthesis (rgg and gtfG). We will evaluate the transcription of abpA, abpB and gtfG in mutant strains to determine if Abp modulates gtfG expression (or vice versa) at the transcriptional level. 2) compare wildtype and mutant strains in standard in vitro adhesion and biofilm models. 3) assess the physical interaction between Gtf and amylase-binding proteins using proteomic approaches such as Western blot-ligand binding assays, Maldi-Tof (Matrix-assisted, Laser-Desorption-Ionization/Time of Flight) mass spectrometry, or phage display. 4) determine if amylase-binding mutations in Gtf-deficient or -proficient *S. gordonii* modulate *S. gordonii* oral colonization and cariogenicity in rats. 5) determine if *S. gordonii*-amylase interactions modulate *S. mutans* colonization competition and cariogenicity and if strains of *S. mutans* made to express AbpA show altered colonization and/or cariogenic abilities. Integration of in vitro with in vivo studies is crucial for mechanistic understanding of Dental plaque formation.



**Grant:** 2R01DE011000-11

**Program Director:** MANGAN, DENNIS F.

**Principal Investigator:** FIVES-TAYLOR, PAULA M PHD  
MICROBIOLOGY:MICROBIOLO  
GY-UNSPEC

**Title:** Genetic Analysis-Adhesion of *S. Sanguis* to *S. Pellicle*

**Institution:** UNIVERSITY OF VERMONT & ST AGRIC BURLINGTON, VT  
COLLEGE

**Project Period:** 1994/05/01-2009/02/28

**DESCRIPTION:** Previous grant support has led to the discovery of a novel *Streptococcus parasanguis* fimbriae associated adhesion, FapI. FapI-like molecules and genes involved in FapI glycosylation are strikingly conserved throughout oral streptococci and are also found in important pathogens, such as *Streptococcus pneumoniae* and *Staphylococcus aureus*. We will use *S. parasanguis* as a model system to study the function of FapI-like proteins in these pathogenic bacteria. FapI is required for the formation of a *S. parasanguis* biofilm. We have determined that the N-terminal polypeptide of FapI (rFapI) is essential for initial attachment of bacteria to the biofilm surface. FapI is glycosylated, and glycosylation of this protein is involved in maturation of biofilm. The overall goal of this application is highlighted by two Specific Aims: (1) to determine the structure/function relationship of the rFapI adhesin that is required for initial bacterial attachment and (2) to define the functions of the fapI flanking genes in FapI glycosylation and bacterial biofilm formation. We will bring genetic, structural biology (NMR spectroscopy and X-ray crystallography), in vitro binding biochemical assays and in vivo animal model studies together to address how the structure of the FapI polypeptide influences its function. These comprehensive approaches should shed light on the structural basis for binding of FapI to salivary receptors, and for predicted FapI-like molecules of other medically important pathogens. Proteins of the fapI flanking region may constitute a coupled secretion and glycosylation pathway for FapI. To begin to understand the mechanisms involved, we will determine the subcellular localization of proteins in this locus and identify putative protein-protein interactions in vivo and in vitro. We previously demonstrated that FapI glycosylation is involved in maturation of the biofilm. Using a combination of molecular and genetic analyses, we will dissect the function of these glycosylation genes in biofilm formation. Elucidation of the function of FapI glycosylation and definition of the FapI polypeptide structure/function relationships will contribute to the field of bacterial colonization in the oral environment and to the pathogenic mechanism of other important pathogens as their genomes indicate that they also possess FapI-like molecules, and the flanking proteins.

**Grant:** 2R01DE011111-11  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** LAMONT, RICHARD J PHD VET  
MEDICINE:MICROBIOLOGY  
**Title:** P.gingivalis Interactions with Gingival Epithelial Cells  
**Institution:** UNIVERSITY OF FLORIDA GAINESVILLE, FL  
**Project Period:** 1995/04/01-2008/12/31

**DESCRIPTION:** *P. gingivalis*, a gram-negative anaerobe is an important component of the oral microbiota. Under certain circumstances *P. gingivalis* can contribute to the initiation and progression of severe and chronic forms of periodontal disease. However, *P. gingivalis* can also inhabit the oral cavity in the absence of overt disease and can engage in a balanced interaction with host cells. Epithelial cells that line the gingival crevice function both as a physical barrier and as sensors of microbial colonization. The outcome of the interaction between *P. gingivalis* and gingival epithelial cells, therefore, makes a significant contribution to the degree of equilibrium between host and microbe, and to overall gingival health status. *P. gingivalis* can manipulate epithelial cell signal transduction pathways in order to direct its entry into the host cell. Internalized *P. gingivalis* remain viable and accumulate in the perinuclear area; and the epithelial cells do not undergo apoptotic or necrotic cell death. However, gene transcriptional activity is modulated in both host and microbial cells. The goal of this proposal is to provide a greater degree of molecular definition to the mechanisms by which *P. gingivalis* and gingival epithelial cells interact and adapt to each other. By constructing specific gene knockouts, the functionality of the genes and proteins differentially regulated by *P. gingivalis* during its association with epithelial cells will be determined. In addition, the means by which *P. gingivalis* impinges upon epithelial cell apoptotic pathways will be investigated. Finally, the transcriptional profile of epithelial cells that are infected with *P. gingivalis* will be studied with microarrays. This system-wide approach will provide novel insights into the eukaryotic cellular physiology that accompanies recognition of *P. gingivalis* and accommodation of intracellular organisms. The ultimate goal of these studies is to use the knowledge gained to develop strategies that could be utilized to intervene in the *P. gingivalis* epithelial cell interaction to ensure that the outcome is non-harmful to the host.

**Grant:** 2R01DE012768-05

**Program Director:** BHARGAVA, SANGEETA

**Principal Investigator:** DARVEAU, RICHARD P  
PHD  
IMMUNOLOGY/IMMUNOPATHOLOGY

**Title:** P. GINGIVALIS LPS-MODULATION OF INNATE HOST DEFENSE

**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA

**Project Period:** 1999/05/01-2009/04/30

DESCRIPTION (provided by applicant): Porphyromonas gingivalis is an important gram-negative periopathogen strongly associated with adult type periodontitis. In addition it is also found to persist in clinically healthy tissue demonstrating an ability to occupy different niches within the host environment. Lipopolysaccharide (LPS), a key component on the cell surface of gram-negative bacteria, is a potent immunomodulator that alerts the host of bacterial infection through a TLR 4 activation pathway. In our previous application we examined some of the unusual innate host defense immunomodulating characteristics of P. gingivalis LPS that suggested this LPS did not always act as a sentry for host recognition. In this first renewal application we propose to continue our studies "to more fully understand the role of P. gingivalis LPS in the innate host immune response." In the present application we will extend our observations and those of others that P. gingivalis LPS displays lipid A structural heterogeneity. Our hypothesis is that: P. gingivalis modifies the number and types of lipid A species present in response to environmental conditions. We suspect that the ability of P. gingivalis to synthesize and express multiple structurally different forms of lipid A represents a form of bacterially induced immunomodulation. This allows the bacterium to selectively evade and stimulate host cell responses in response to local environmental conditions contributing to its ability to occupy different niches in the host. To examine the hypothesis we have constructed three Specific Aims. The first Aim will develop techniques for the further characterization of multiple lipid A species in P. gingivalis and examine environmental factors in their regulation. The second Aim will examine the genetic mechanisms of how P. gingivalis lipid A diversity is generated. The third Aim will examine potential TLR mediated innate host interactions of the different lipid A species generated in Aims 1 and 2.

**Grant:** 2R01DE013819-04  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** CHU, LIANRUI MD  
**Title:** H2S production and virulence of *Treponema denticola*  
**Institution:** UNIVERSITY OF TEXAS HLTH SCI CTR SAN SAN ANTONIO, TX  
ANT  
**Project Period:** 2000/09/01-2007/12/31

**DESCRIPTION:** This application is an extension of current research examining the association between H2S production and virulence of *Treponema denticola*. *T. denticola* has been identified as an important member of a consortium of microorganisms as etiologic in the initiation and progression of periodontal diseases. In addition, the existence of volatile sulfur compounds produced at destructive sites is a characteristic feature of periodontal diseases, with H2S as a major compound in this family. Nevertheless, the metabolic pathways used to produce H2S are not well understood. Previous studies suggest that glutathione present in host cells can be a substrate for H2S production. Recently, we have shown that *T. denticola* has the capacity to utilize glutathione as a substrate to produce high levels of H2S. We have identified three enzymes that are required for the successful metabolism of glutathione: gamma-glutamyltransferase (GGT), cysteinylglycinase (CGase), and cystalysin. GGT converts glutathione into Cys-Gly and glutamic acid; CGase catalyze Cys-Gly to Cys and glycine; and cystalysin digests L-cysteine into H2S, ammonia, and pyruvate. We have also demonstrated that the addition of cystalysin and L-cysteine resulted in apoptosis of HGF and PDL cells and that glutathione was essential for lesion formation by *T. denticola* in an animal model. Based on these and other studies, three Specific Aims are proposed using biochemical, molecular genetic, and cell biologic studies to address the hypothesis that these three metabolic enzymes play a key role in *T. denticola* pathogenesis. Specific Aim 1: To molecularly characterize the genes and proteins involved in converting glutathione to H2S. Specific Aim 2: To genetically characterize, by gene inactivation, the enzyme pathway of *T. denticola* that produces H2S from glutathione. Specific Aim 3: To measure the effects of the H2S/NH3 producing pathway on *T. denticola* virulence in vitro and in vivo. This application is designed to provide both seminal and critical information about the enzyme pathway of *T. denticola* to produce H2S from glutathione. The outcomes will elucidate the mechanisms of action that each member of the enzyme pathway plays in the virulence capacity of *T. denticola*.

**Grant:** 2R01DE013824-04  
**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:** 2000/07/01-2006/06/30

**DESCRIPTION:** *Actinobacillus actinomycetemcomitans* is a Gram-negative, facultative anaerobic bacterium that colonizes the human oral cavity and the upper respiratory tract. This bacterium is strongly associated with localized aggressive periodontitis (LAP) and with cases of adult periodontitis. This pathogen is the causative agent for other serious infections including infectious endocarditis, soft tissue abscesses, and pneumonia and may contribute to cardiovascular disease. The periodontium is believed to be the source for these non-oral diseases, but little is known about the tropism used by *A. actinomycetemcomitans* to colonize the oral cavity and to infiltrate and disseminate in tissues. Pathogens have developed diverse strategies to be successful in colonization of host tissues. 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 secreted by host cells that form the extracellular matrix (ECM). *A. actinomycetemcomitans* is found in the connective tissue of the periodontium and in close association with collagen fibers in infected tissues. The bacterium also binds to the ECM proteins, collagen, fibronectin and laminin. Using a genetic approach, we have identified the first *A. actinomycetemcomitans* collagen adhesin, Ema (extracellular matrix protein adhesin)A and multiple genes involved in regulating the expression of ECM protein adhesin activity. EmaA is structurally related to YadA, a multipurpose ECM protein adhesin of the enteropathogenic bacterium *Yersinia enterocolitica*. To elucidate the role of EmaA in colonization and pathogenicity of the bacterium, we propose to 1) investigate the protein domains of EmaA in terms of cellular localization and collagen binding, 2) investigate the surface structures associated with EmaA and the in vitro virulence potential of this protein, and 3) determine the in vivo virulence of *emaA* in a rat endocarditis and mouse periodontitis model. A long term goal of the proposed research is to identify and characterize bacterial adhesins that are required for the colonization of the oral cavity and non-oral tissues. These adhesins may serve as targets for future drug development involving small molecules or vaccines that disrupt host-pathogen interactions.

**Grant:** 1R01DE014371-01A2  
**Program Director:** BHARGAVA, SANGEETA  
**Principal Investigator:** COSTALONGA, MASSIMO PHD  
**Title:** Tracking Mucosal T cells to Commensal Microbes in Vivo  
**Institution:** UNIVERSITY OF MINNESOTA TWIN CITIES MINNEAPOLIS, MN  
**Project Period:** 2004/02/01-2007/01/31

**DESCRIPTION:** IgA antibodies provide an important first line of defense against mucosal pathogens. The induction of immunoglobulins against T cell-dependent antigens is subordinate to T cell activation, T/B cell interaction and cytokines. Our long-range goal is to learn how the mucosal immune system responds to protein antigens of the virulent and commensal microbiota. Our current objective is to determine, in vivo, which type of T cell response is induced by the commensal *Lactobacillus murinus*, while transiting the intestine. We hypothesize that intestinal commensal microorganisms transmucosally induce a T cell-dependent humoral response, while suppressing the cell-mediated response. Testing this hypothesis can lead to the design of effective microbial delivery systems. We will test this hypothesis by determining 1) how a commensal microbe primes naive T cells in the intestine and 2) the type of cytokines elicited in memory T cells. We engineered *L. murinus* to express an ovalbumin epitope that induces ovalbumin-specific T cell proliferation when injected subcutaneously. We inject a small population of ovalbumin-specific T cells into recipient mice and feed *L. murinus* in high numbers. Ovalbumin-specific T cells are tracked by staining cell suspensions or tissues of the recipients with anti-CD4 and an anti-T cell receptor monoclonal antibody. Four-color flow cytometry and confocal immunohistology will establish in vivo that commensal microorganisms transiting the intestine activate T cells to induce CD69 expression. In an antigen-specific manner we will test the phenotype and kinetics of transmucosal T cell activation, proliferation and differentiation into cytokine-producing memory cells. Collectively, this research will elucidate the missing link between the oral antigen delivery and the production of secretory antibodies. The data will be important to human health, establishing a framework to study in vivo mucosal infectious agents and oral vaccines.

**Grant:** 1R01DE014685-01A2  
**Program Director:** MOWERY, RICHARD L  
**Principal Investigator:** SCANNAPIECO, FRANK A. DMD  
**Title:** Oral Health and Ventilator Associated Pneumonia  
**Institution:** STATE UNIVERSITY OF NEW YORK AT AMHERST, NY  
BUFFALO  
**Project Period:** 2004/07/01-2007/06/30

DESCRIPTION (provided by applicant): Recent studies have found that poor oral hygiene may foster the colonization of the oropharynx by potential respiratory pathogens in mechanically-ventilated (MV), intensive care unit (ICU) patients. Thus, improvements in oral hygiene in MV-ICU patients may prevent ventilator-associated pneumonia (VAP). The Specific Aims of this revised application are: 1) to organize the necessary infrastructure to develop and perform a pilot clinical trial to evaluate alternative oral hygiene procedures to prevent VAP; 2) to use this organization to perform a pilot clinical trial to determine if the use of oral topical chlorhexidine gluconate (CHX) will prevent dental plaque, oropharyngeal colonization by respiratory pathogens, and VAP in MV-ICU patients. This pilot longitudinal, double blind intervention study will consider the appropriate frequency of delivery of CHX to improve oral hygiene in MV-ICU patients. Preliminary data from these pilot studies will also allow accurate sample size calculations to be made for a large scale multi-center clinical trial; and 3) to perform molecular epidemiological studies to genetically type bacterial strains cultured from lower airway secretions of MV-ICU patients with or without VAP and compare them to strains of the same species isolated from their dental plaque. This pilot study will enable this multidisciplinary team of investigators to organize the infrastructure, patient recruitment and methodologic protocols, and data management and analysis procedures necessary to perform a multi-center, controlled clinical trial to assess the efficacy and generalizability of this intervention to improve oral hygiene in MV-ICU and prevent VAP.

**Grant:** 1R01DE014699-01A2  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** XIE, HUA MS  
**Title:** Intergeneric Signaling Molecules of *S. cristatus*  
**Institution:** MEHARRY MEDICAL COLLEGE NASHVILLE, TN  
**Project Period:** 2004/01/01-2008/11/30

**DESCRIPTION:** Dental plaque (biofilm) has been implicated as a primary causative agent of adult periodontal disease. The key event leading to initiation of the disease is the transition from commensal Dental biofilm to pathogenic biofilm. It is well known that the process of the transition is involved in the colonization of several specific periodontal pathogens such as *Porphyromonas gingivalis*. The long-range goal is to understand events and factors leading to the transformation of healthy plaque to pathogenic plaque and to change the course of development of periodontopathogenic biofilm by preventing attachment of *P. gingivalis*. In our ongoing studies, we have identified several environmental factors that can influence expression of *fimA* gene, a virulence gene encoding a major protein unit (fimbriin) of fimbriae. One of the striking findings is that the presence of *Streptococcus cristatus* molecule(s) could significantly repress *fimA* expression in *P. gingivalis* at the transcriptional level. As a result, *S. cristatus* could inhibit the formation of *P. gingivalis* biofilm in vitro. In this grant application, we will put our focus on characterization of *S. cristatus* signaling molecule, biochemically and genetically. The hypothesis for this application is that *S. cristatus* plays an important role in impeding *P. gingivalis*' colonization on Dental biofilm through intergeneric signaling systems. To test this hypothesis, we will start with identification and purification the signaling molecule(s) of *S. cristatus*. The signaling molecule will be characterized in the terms of functional and genetic structures. We will also attempt to understand regulation of the signaling gene expression in oral biofilm. Therefore, the signaling gene of *S. cristatus* will be cloned. The promoter region of the gene will be fused with the reporter gene such as chloramphenicol acetyltransferase gene, and level of the gene expression will be determined by measuring enzymatic activity. Finally the role of this molecule in the formation of pathogenic oral biofilm will be investigated. Studies will be initiated to determine the distribution of the signaling molecule in the Dental plaques from healthy subjects and periodontitis patients. Our ultimate goal is to convert the knowledge gained from these laboratory studies to practical technology that may be used to reprogram development of the Dental biofilm and to reduce the incidence of adult periodontitis.



**Grant:** 1R01DE014711-01A2  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** SPATAFORA, GRACE A. PHD BIOLOGY  
**Title:** Cloning/Characterization of *S. mutans* Iron Stimulation  
**Institution:** MIDDLEBURY COLLEGE MIDDLEBURY, VT  
**Project Period:** 2004/07/15-2009/04/30

**DESCRIPTION** (provided by applicant): Iron is an essential micronutrient for bacterial pathogens and their mammalian hosts, and hence competition for this element is an integral component of the infectious process. Extracellular ferrous iron is biologically unavailable to invading pathogens because it is largely sequestered to host proteins, and in its free form rapidly oxidizes to the insoluble ferric state. Bacteria often respond to the iron withholding system of the host by turning on iron scavenging systems and virulence determinants to promote bacterial survival and dissemination. Among the most extensively studied metal ion-dependent regulators in Gram-positive bacteria is DtxR, a DNA-binding protein, in *Corynebacterium diphtheriae*, and its phylogenetic homologs SirR in *Staphylococcus epidermidis* and MntR in *Staphylococcus aureus*. Importantly, these metalloregulators function as transcription factors that modulate the expression of bacterial virulence genes and high affinity metal ion uptake systems related to virulence and oxidative stress. Work conducted in our laboratory on *Streptococcus mutans*, the principal acidogenic component of human dental plaque, revealed a DtxR homolog that regulates a repertoire of genes, and accumulating evidence is consistent with a role for iron in *S. mutans* biofilm formation, the oxidative stress response, and acid tolerance. Indeed, the identification and characterization of genes involved in these and other pathogenic processes is a high priority since they can prove to be future drug targets. The major goal of this research proposal will focus on elucidating iron-dependent gene regulation and its role in *S. mutans*-induced cariogenesis. The specific aims include 1) characterizing iron-responsive genes that belong to a putative *S. mutans* Dlg regulon; 2) cloning and characterizing other iron-dependent genes in *S. mutans* that are not subject to Dlg control; 3) examining the expression of *S. mutans* Dlg- and iron-responsive genes in environments that approximate the human oral cavity; and 4) establishing a correlation between Dlg/iron-regulated gene expression and *S. mutans*-induced cariogenesis.

**Grant:** 1R01DE014868-01A2  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** GILL, STEVEN R PHD  
**Title:** Community Genomics of the Human Oral Microbiome  
**Institution:** INSTITUTE FOR GENOMIC RESEARCH ROCKVILLE, MD  
**Project Period:** 2004/08/16-2007/07/31

**DESCRIPTION:** The human oral cavity is home to a complex community of >700 microorganisms with a central role in oral health and disease. In our application, this oral microbial community, or oral microbiome, is viewed as a single dynamic entity, with variation in its behavior and composition across space (different oral niches in different individuals), time, and with perturbation. Despite the critical importance of this community in maintaining human health and provoking oral disease, the genetic basis for its behavior has not been well explored. The long-term objectives of this application are to develop a comprehensive understanding of the oral microbial community at the genomic level so that oral health can be promoted, and disease ameliorated or prevented. We will address these goals by characterizing relative gene and genome abundance and gene expression in the oral microbiome in subjects with healthy periodontal and mucosal tissues, and then extend our analysis to the oral microbiome associated with chronic periodontal diseases. Data from this application will not only be used to build a database for genes in the oral microbiome, but will also lead to new experimental paradigms for exploring gene abundance and expression, and examining the interaction of the microbiome with the human host. The Specific Aims of this application are: Aim 1. Characterize the gene and genome content of the healthy human oral microbiome. Our approaches include high-throughput sequencing of random and targeted plasmid and fosmid genomic libraries and classification of functional genes and gene families. Aim 2. Characterize the gene and genome content of the oral microbiome associated with chronic periodontitis. Aim 3. Development of community genomic tools for oral microbiome data. An oral microbiome gene database will be created for use by the scientific community. A high-density DNA microarray with approximately 2,000 genes identified in Aims 1 and 2 will be developed with the ultimate goal of identifying patterns of gene abundance and expression that predict clinical natural history and response to manipulation. Among the expected long-term benefits of this work will be a revolutionized understanding of the human oral ecosystem and new approaches for classification and management of oral diseases.

**Grant:** 1R01DE015124-01A2  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** KAPLAN, JEFFREY B PHD  
**Title:** Biofilm growth and detachment of an oral pathogen  
**Institution:** UNIV OF MED/DENT NJ NEWARK NEWARK, NJ  
**Project Period:** 2004/07/01-2008/03/31

**DESCRIPTION:** Biofilms are communities of bacteria growing attached to a surface. Biofilms are responsible for more than 80% of bacterial infections in humans. Examples of diseases caused by biofilms include Dental caries, periodontitis, cystic fibrosis pneumonia, and infective endocarditis, and infections of various medical devices such as intravenous catheters, artificial joints and contact lenses. Little is known about the detachment of bacteria from biofilms, a process necessary for the spread of infections to new sites. Biofilm detachment represents an important area of future research that is expected to lead to novel strategies for treating biofilm infections. The Gram-negative oral bacterium *Actinobacillus actinomycetemcomitans* has been implicated as the causative agent of localized juvenile periodontitis, a severe and rapid form of periodontal disease that affects 70,000 primarily African-Americans in the U.S. annually. *A. actinomycetemcomitans* also causes several non-oral infections including bacteremias, brain abscesses and infective endocarditis. A striking feature of fresh clinical isolates of *A. actinomycetemcomitans* is their ability form extremely tenacious biofilms on surfaces such as glass, plastic and saliva-coated hydroxyapatite, a property that has been shown to be essential for virulence in a rat model. Tight adherence to surfaces also makes *A. actinomycetemcomitans* an excellent model for studying biofilm growth and detachment in vitro. Genetic and microscopic studies in this laboratory have shown that *A. actinomycetemcomitans* cells grown attached to surfaces in broth form highly-differentiated biofilm colonies that are capable of releasing cells into the medium. Biochemical and genetic studies indicate that *A. actinomycetemcomitans* biofilm colonies are held together by a sticky, extracellular polysaccharide. The proposed experiments are a continuation of our preliminary studies which have identified an enzyme produced by *A. actinomycetemcomitans* which causes the degradation of the sticky polysaccharide coating and detachment of *A. actinomycetemcomitans* cells from the biofilm aggregate. We plan to use genetic techniques to understand how production of this enzyme is regulated in the bacterial cell, and biochemical techniques to determine the structure of the polysaccharide substance on the surface of the cell. Preliminary data indicate that this enzyme is capable of degrading biofilms produced by other species of Gram-negative and Gram-positive bacteria, indicating that it may represent a novel anti-biofilm therapeutic with broad spectrum potential.

**Grant:** 1R01DE015254-01A2  
**Program Director:** BHARGAVA, SANGEETA  
**Principal Investigator:** HAJISHENGALLIS, GEORGIOS DDS DENTISTRY  
**Title:** Pattern Recognition of *P. gingivalis* Virulence Factors  
**Institution:** LOUISIANA STATE UNIV HSC NEW ORLEANS NEW ORLEANS, LA  
**Project Period:** 2004/07/01-2008/06/30

**DESCRIPTION:** Toll-like receptors (TLRs) and other pattern-recognition receptors (PRRs) form functional receptor complexes that recognize pathogen-associated molecular patterns (PAMPs). Activation of the TLR signaling pathway by PAMPs leads to induction of immune and inflammatory responses. *Porphyromonas gingivalis* is an important pathogen in human periodontitis. A major cell surface component of this oral pathogen is the fimbriae, which function as an adhesin. Strikingly, fimbriae activate transcription factor NF-KappaB and induce production of proinflammatory cytokines through interactions with several PRRs. Understanding the molecular basis of how the host recognizes and responds to *P. gingivalis* fimbriae is essential for developing molecular approaches to control periodontal inflammation. Therefore, the objective of this grant is to elucidate the proinflammatory interactions of fimbriae with PRRs. The application proposes that fimbriae function as a PAMP and interact in a regulated mode, and through discrete epitopes, with different binding PRRs resulting in the activation of proinflammatory TLR signaling. Fimbrial epitopes involved in cellular binding and/or activation will be identified using fimbrial peptides and mutant fimbriae. Importantly, epitopes involved in binding but not activation may find application as antagonists of fimbria-induced inflammation. Experiments in our laboratory have shown that TLR2 and TLR4 mediate fimbria-induced signaling, but initial recognition of fimbriae is mediated by a cooperation between CD14 and CD11 b/CD18, which thus appear to serve as TLR co-receptors. It is posited that fimbriae initially bind to CD14, and the fimbriae/CD14 complex induces TLR2-mediated "inside-out" signaling that leads to activation of the ligand-binding capacity of CD11b/CD18. PRR-fimbriae interactions will be examined in human monocytes and mouse macrophages derived from normal and PRR-deficient mice. Elucidation of the mechanisms whereby PRRs recognize and respond to fimbriae and identification of fimbrial antagonists may facilitate the design of novel approaches to therapeutic intervention in both periodontitis and atherosclerosis, where *P. gingivalis* has also been implicated.

**Grant:** 1R01DE015354-01A1  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** LUKEHART, SHEILA A  
**Title:** INTERACTION OF ORAL SPIROCHETES WITH GINGIVAL EPITHELIUM  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 2004/03/01-2007/11/30

DESCRIPTION (provided by applicant): Oral spirochetes, including *Treponema denticola*, comprise about 40 percent of the bacteria in periodontal pockets and are linked to the progression and severity of periodontal disease. Despite their obvious relevance to periodontal disease, they are vastly understudied. Oral spirochetes are usually found in close association with the gingival epithelium, but our understanding of how *T. denticola* and other oral spirochetes establish themselves at this interface is limited. *T. denticola* and other oral spirochetes likely have evolved strategies to avoid host immune defenses, specifically the defenses initiated by the epithelial cell. The proposed studies will focus on the interactions of spirochetes with gingival epithelial cells and the modulation of molecules produced by these cells in response to bacterial challenge. Our studies to date demonstrate that *T. denticola* is resistant to  $\alpha$ -defensins, and the susceptibility of other oral treponemes to beta-defensins will be examined (Aim 1). To define the mechanisms used by *T. denticola* to resist beta-defensins killing, we will explore strategies used by other bacteria to resist antimicrobial peptides, including bacterial proteases, binding of defensin to cell surfaces, and efflux pumps (Aim 2). The ability of *T. denticola* and other oral treponemes to induce production of beta-defensins will be examined in Aim 3. Our preliminary data suggest that *T. denticola* inhibits the induction of inflammatory cytokines by other bacterial products, similar to the "chemokine paralysis" that has been described for *Porphyromonas gingivalis* LPS. We will examine the modulation of cytokines and adhesion molecules from epithelial cells stimulated by *T. denticola* (Aim 4), with the goal of identifying the signaling pathways that are affected by the spirochetes. Lastly, we will determine the components of *T. denticola* (Aim 5) that induce production of  $\alpha$ -defensins and antagonize the induction of inflammatory cytokines. This study will further our understanding of the pathogenesis of periodontal disease by providing a more complete understanding of how *T. denticola* and other oral spirochetes evade the innate immune response.

**Grant:** 1R01DE015517-01  
**Program Director:** NOKTA, MOSTAFA A  
**Principal Investigator:** SELSTED, MICHAEL E MD  
**Title:** Molecular Ontogeny of Oral Mucosal Resistance to SIV  
**Institution:** UNIVERSITY OF CALIFORNIA IRVINE IRVINE, CA  
**Project Period:** 2003/12/15-2007/11/30

Epidemiologic studies demonstrate that the oral cavity of human adults is relatively resistant to HIV infection, and a number of soluble factors present in saliva have been postulated to confer this protection. In contrast, oral infection of infants who are breast fed by HIV-infected mothers is quite common, suggesting that there are age dependent differences in oral resistance to lentiviruses. We will investigate the basis of these age-dependent differences in antiviral resistance by characterizing anti-SIV innate immunity in the oral cavity of rhesus macaques. The long term goal of the proposed studies is to delineate the anti-HIV role of defensins, antiviral peptides now known to be present in saliva and expressed in epithelium of the oral cavity. Three human defensins were recently identified as anti-HIV factors produced by CD-8+ T cells from HIV-positive individuals who are long term non-progressors. We hypothesize that defensins contribute to the anti-SIV/HW properties of saliva and to the innate resistance of oral mucosa, 2) that oral defensin expression develops postnatally, and 3) that the level of oral resistance to SIV in neonates may be augmented by topical application of one or more defensins. To test these hypotheses, we will pursue the following Specific Aims: ? Specific Aim 1 is to determine the level of alpha, beta, and theta-defensins present in saliva and/or expressed in the oral cavity of infant and adult rhesus macaques. ? Specific Aim 2 is to synthesize and/or recombinantly express specific alpha, beta, and theta-defensins confirmed (in Specific Aim 1) to be components of adult saliva and/or expressed in oral tissues. Peptides thus produced will be fully characterized and evaluated for their anti-SIV efficacy (Specific Aim 3), and will be used to produce anti-peptide immunologic reagents. ? Specific Aim 3 is to determine the anti-SIV and anti-HIV activities of oral alpha, beta, and theta-defensins in vitro. Anti-HW assays will be conducted with and without neonatal and adult saliva to ascertain the effect of this natural fluid on peptide activities. Combinations of peptides will also be analyzed to detect additive or synergistic peptide-peptide interactions. ? Specific Aim 4 is to determine whether exogenous, topically administered defensin can alter the susceptibility to infection of neonatal rhesus macaques.

**Grant:** 1R01DE015594-01A1

**Program Director:** CANTO, MARIA TERESA

**Principal Investigator:** DASANAYAKE, ANANDA P DDOT BIOPHYSICS:ATOMIC  
STRUCTURE

**Title:** Periodontal Disease and Prematurity

**Institution:** NEW YORK UNIVERSITY NEW YORK, NY

**Project Period:** 2004/08/01-2007/04/30

**DESCRIPTION:** Babies who are born prematurely and/or are underweight at birth are 20-80 times more likely to die before their first birthday. A significant proportion of preterm deliveries occur in women who are free of known risk factors. Periodontal disease is emerging as a potential risk factor although the results from human studies are equivocal. The long-term objective of this research is to further evaluate the extent to which the infections of periodontal origin play a role in preterm delivery. The primary Specific Aims of this project are 1) to measure the association between periodontal pathogens in cervical and vaginal samples of pregnant women and preterm birth, and 2) to measure the association between prenatal maternal serum IgG levels against specific periodontal pathogens and preterm birth. These Aims will be accomplished by conducting a nested case-control study within a large ongoing cohort study at the New York University Medical School (March-of-Dimes Study). Frozen serum and cervico-vaginal samples, data on biochemical markers such as IL-6, IL-8, TNFalpha, and Thrombin-antithrombin complexes, and validated questionnaire data on quality-of-life are available from this study. As of October 1,2003, there were 1579 subjects enrolled in this study and 1054 have already delivered (7% preterm deliveries and 10% low birth weight). Sixty-nine percent were Hispanic and 85% were 19-34 years of age. This study has two more years of enrollment remaining with an estimated 215 new recruits per year. Accordingly, we estimate that by October 2005 there will likely be 140 preterm and 1869 full term deliveries. Three pair-matched controls for each preterm delivery case will be selected from the full-term delivery subjects based on age, race, and date of delivery. Periodontal pathogens will be measured in cervico-vaginal samples using a checkerboard assay, and periodontal pathogen-specific IgG levels will be measured in serum samples using ELISA. Conditional logistic regression analysis will be used in the data analysis. As a secondary Aim, on an exploratory basis, levels of periodontal pathogens in oral, cervical, and vaginal samples, and periodontal pathogen specific serum IgG levels will be correlated with each other and also with preterm birth using fresh samples collected from the subjects that will be recruited to the March-of-Dimes Study after this application is funded (October 04-Oct 05; N=215). The proposed research is important because it will help identify the nature and the strength of the association between preterm delivery and periodontal disease-related factors which are modifiable in comparison to a majority of obstetrical risk factors that are not as easily changed. These associations have potential application in the early diagnosis and prevention of preterm deliveries. A major strength of the study is the efficacy gained by utilizing existing data and samples from a large cohort study. The collective expertise of the investigative team and the excellent clinical and research facilities are additional strengths.

**Grant:** 1R01DE015625-01A1  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** KOLODRUBETZ, DAVID J PHD  
**Title:** Anaerobic Regulatory Pathways in a Periodontopathogen  
**Institution:** UNIVERSITY OF TEXAS HLTH SCI CTR SAN SAN ANTONIO, TX  
ANT  
**Project Period:** 2004/07/01-2007/03/31

**DESCRIPTION:** Periodontitis, an inflammatory disease of tissues in the subgingival crevice, is associated with a dramatic shift in the subgingival microflora towards Gram negative organisms. We have focused on studying the virulence properties of one of these periodontal pathogens, the bacterium *Actinobacillus actinomycetemcomitans* (Aa). This bacterium has been strongly implicated in localized aggressive periodontitis and in several adult periodontal disorders. Aa is a facultative anaerobe that is capable of colonizing both an anaerobic microenvironment, like the diseased periodontal pocket, and an aerobic environment, like the blood stream. Thus, Aa has evolved virulence mechanisms that allow it to shift between these two environments. Using two-dimensional protein gels, we have shown that anaerobic growth induces the synthesis of dozens of Aa proteins, including leukotoxin (a presumed virulence protein that kills neutrophils). Numerous other proteins are repressed under anaerobic conditions but induced in the presence of oxygen. By generating defined mutations in An, we have shown that the aerobic/anaerobic regulation of most of these proteins is controlled by the Fnr or ArcAB pathways, as expected. Nevertheless, interestingly, the regulation of at least eight other proteins, including leukotoxin, was not controlled by Fnr or ArcAB. Since Aa does not encode significant homologues to any other bacterial oxygen regulatory proteins, we posit that a subset of Aa genes will be controlled by one (or more) transcriptional regulatory pathways which have not previously been described in Aa or in any other bacterial system. The full complement of Aa genes that are differentially synthesized in aerobic versus anaerobic growth will be identified using DNA microarrays (Specific Aim I). Importantly, we will also identify the set of oxygen-responsive genes, like leukotoxin, that are regulated by the previously unidentified, non-Fnr/non-ArcA pathway(s). In Specific Aim II, a systematic and comprehensive molecular genetic approach will be employed to identify this potentially novel regulatory pathway and the transcription factors involved. Finally, the interactions of our newly identified oxygen regulatory proteins with redox-regulated Aa promoters will be characterized (Specific Aim III). This will allow us to begin to develop the first molecular models for the mechanisms of non-Fnr/non-ArcAB aerobic/anaerobic regulation in a periodontal pathogen. The research proposed is highly significant because it will reveal the players in a new regulatory pathway regulating the adaptation of An, and possibly other periodontal pathogens, to the anaerobic subgingival microenvironment. Characterizing undefined, potentially new transcriptional regulatory pathways is important because the proteins involved may serve as targets for future drug development.



**Grant:** 1R01DE015663-01A1  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** MACRINA, FRANCIS L  
PHD  
GENETICS:BIOCHEMICAL/MOL  
ECULAR  
**Title:** Novel Plasmids for Porphyromonas Post-Genomic Research  
**Institution:** VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA  
**Project Period:** 2004/07/01-2007/03/31

**DESCRIPTION:** Porphyromonas gingivalis is recognized as a prime etiological agent in periodontitis, a destructive disease of the supporting structures of the teeth which is prevalent in adults. Molecular genetic research on P. gingivalis has begun to build a foundation of understanding concerning how this organism colonizes the oral cavity and causes pathology. A dramatic increase in this understanding is likely now that the nucleotide sequence of the genome of P. gingivalis W83 has been determined. To date, genetic analyses of this organism have been possible using a number of tools adapted from other bacterial systems. A few genetic tools have been specifically created for use in Porphyromonas sp., but the systematic construction of plasmid vectors is critically needed if we are to fully exploit the genomic nucleotide sequence of this pathogen. We propose to accomplish this by developing, testing, and distributing plasmid constructs needed for the in-depth postgenomic analyses of Porphyromonas gingivalis. Specifically, we shall: 1. Characterize and native and recombinant plasmids from oral black pigmented Gram negative anaerobes for their ability to stably support extrachromosomal replication in P. gingivalis; 2. Design, build, characterize and test several robust shuttle vectors for the analysis of P. gingivalis; 3. Using 4 different non-clonal P. gingivalis strains, evaluate cellular and molecular variables that may affect their suitability as hosts for molecular genetic analyses; 4. Distribute these new genetic tools and knowledge about their use to workers studying Porphyromonas gingivalis and other black pigmented Gram-negative anaerobes.

**Grant:** 1R01DE015720-01  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** KESAVALU, LAKSHMYA N DVM  
**Title:** Oral Pathogens: Polymicrobial Virulence Interactions  
**Institution:** UNIVERSITY OF KENTUCKY LEXINGTON, KY  
**Project Period:** 2004/04/01-2006/12/31

**DESCRIPTION:** The predominant polymicrobial infection of mankind is expressed clinically as periodontal disease, which afflicts nearly one-half of the population by 50 years of age, and is related to development of a microbial biofilm colonizing the subgingival sulcus. The suggested mechanisms of pathogenesis are varied, in most part due to the complex microbial community consisting of numerous bacterial taxa, viruses, and fungi. Nevertheless, certain of these subgingival microbial consortia are consistently correlated with a progressive destruction of soft and hard tissue that have been well documented to occur in clinical settings (i.e., periodontitis). Various in vivo and in vitro investigations have suggested that the dominance of selected species in the subgingival ecology results from both microbial synergistic and antagonistic relationships. These have been linked to the nature of available surfaces for colonization, available nutrients, and physiologic "food webs" that exists within the community. Molecular microbiologic studies have described nearly 500 species of bacteria that can inhabit this ecological niche, although several specific microbial complexes have been described at sites of progressing tissue destruction. A predominant consortia identified in a majority of adult periodontitis patients consists of *Porphyromonas gingivalis*, *Tannerella forsythensis* [*Bacteroides forsythus*], and *Treponema denticola*. The correlation of this consortium with disease has been proposed to result from synergistic physiological, host evasion, and/or tissue destructive capabilities among the component species. The objectives of this R01 application are to test a hypothesis that this polymicrobial consortium comprises a "virulence web" that synergistically increases tissue destructive host responses, and the consortia to be less effective modify that host immune responses. Three Specific Aims are proposed using an animal model system to test this hypothesis: (1) To determine molecular interbacterial synergistic virulence effects of *P. gingivalis*, *T. forsythensis*, and *T. denticola* in an in vivo calvarial bone resorption model, (2) To determine the characteristics of acquired humoral immune responses to a polymicrobial infection and the ability of this response to modulate in vivo calvarial bone resorption, and (3) To determine the characteristics of active humoral immune responses to polymicrobial immunization and ability of this response to modulate bone resorption. The long-range goals from this study will be to document microbial interactions, virulence synergisms, characterize both acquired and active immune responses, and relate these to alterations in tissue destruction and bone resorption. The significance of this application is that clinical observations have shown the ability of oral microorganisms to translocate into the circulation and manifest systemically as endocarditis, brain/kidney/lung, and intra-abdominal infections and contributing to risks of diabetes, coronary artery disease, osteoporosis, obesity, and preterm birth. Consequently, the host response to these chronic infections must be considered as critical to general health.

**Grant:** 1R01DE015931-01  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** DUNCAN, MARGARET J PHD  
**Title:** Genomics of Gene Regulation in *Porphyromonas gingivalis*  
**Institution:** FORSYTH INSTITUTE BOSTON, MA  
**Project Period:** 2004/04/01-2007/12/31

DESCRIPTION (provided by applicant): Deciphering the "conversation" between bacterium and host during infection is central to understanding microbial pathogenesis and host defense. Periodontal diseases have long been recognized as bacterial infections, and a long-term goal of the laboratory is to identify *P. gingivalis* genes expressed during infection, ie. one side of the cross-talk between bacterium and host. The genetic tractability of the organism, combined with the availability of a completed genome sequence and gene microarrays, has made this goal approachable. Bacteria use two-component signal transduction systems to monitor and react to changes in their environment. In this application, we test the hypothesis that in *P. gingivalis* these systems are responsive to in vivo conditions prevailing in the subgingival crevice during health and disease. A comprehensive study is proposed of the molecular mechanisms of gene regulation in *P. gingivalis*, a new area of research with this organism. Response regulators will be analyzed to identify the genes they control, and their promoter targets. We will test our hypothesis by determining the expression of a subset of identified genes in the in vivo environment of subgingival plaque from periodontitis patients. The experimental approaches exploit our experience in molecular genetics, whole genome transcription profiling, promoter target localization, and DNA sequence analysis. The goals are responsive to the RFA since we will analyze *P. gingivalis* intracellular signaling mechanisms that orchestrate responses to environmental changes. By defining the regulators, the genes they control, and their in vitro and in vivo expression patterns, we can identify conditions that may trigger periodontitis, and target rational therapeutic interventions to the regulatory molecular switches.

**Grant:** 1R01DE015973-01  
**Program Director:** BHARGAVA, SANGEETA  
**Principal Investigator:** WANG, CUN-YU  
**Title:** Induction of intergrating NF-kB by P gingivalis LPS  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2004/04/01-2007/12/31

**DESCRIPTION** (provided by applicant): The long-term objective of this application is to understand the role of NF-kB (nuclear factor kappa B) in the molecular pathogenesis of periodontal diseases using genomic and proteomic approaches. NF-kB is a transcription factor which regulates a variety of immediate early response genes associated with inflammation, immunity and host responses. Periodontal diseases are chronic Gram-negative anaerobic bacterial infections leading to inflammation of the gingiva and destruction of periodontal tissues. Several bacteria including *Porphyromonas gingivalis* (*P. gingivalis*) have been implicated in the initiation and exacerbation of periodontitis. LPS, a major component of the outer membrane of these bacteria, is one of the most potent initiators of host inflammatory and immunological response which results in destruction of periodontal supporting tissue. Recently, the Toll-like receptor (TLR) complex which transduces LPS signaling has been identified. LPS/TLR interaction transduces signaling cascades to activate NF-kB which turns on transcription of inflammatory mediators. Although the TLR signaling complex has been well characterized, the precise mechanisms of NF-kB activation and signaling have not been well studied. Given the critical role of NF-KB in inflammation and host response, NF-kB is likely to play an important role in the molecular pathogenesis of periodontitis. To better understand the role of NF-kB in the molecular pathogenesis of periodontitis, we propose to globally dissect NF-kB-mediated genes and intracellular signaling pathways stimulated by *P. gingivalis* LPS using genomic and proteomic approaches. In Aim 1 and Aim 2, we propose to identify *P. gingivalis* LPS-induced genes regulated by the canonical and non-canonical NF-kB signaling pathways on a genome wide basis and explore how the NF-kB-dependent transcription is regulated using genomic and proteomic approaches. In Aim 3, we will determine whether the canonical and non-canonical NF-kB signaling pathways are activated in inflamed periodontal tissues and explore whether their activation is associated with the gene expression profile induced by *P. gingivalis* LPS using tissue microarray and protein array. The novel findings from our studies will have important implications in the prevention, diagnosis and treatment of periodontal diseases.

**Grant:** 1R01DE015989-01  
**Program Director:** BHARGAVA, SANGEETA  
**Principal Investigator:** AMAR, SALOMON PHD  
**Title:** Genomics Periodontal Host-Parasite Interactions  
**Institution:** BOSTON UNIVERSITY MEDICAL CAMPUS BOSTON, MA  
**Project Period:** 2004/03/01-2007/11/30

DESCRIPTION (provided by applicant): Periodontitis is an inflammatory disease with host-parasite interactions known to contribute to connective tissue destruction and alveolar bone resorption the landmark of this disease. *Porphyromonas gingivalis* (P.g.), a black-pigmented Gram-negative anaerobic bacterium, has been implicated as a major periodontal pathogen in the development and progression of periodontal disease. Structures on its bacterial cell wall and appendage, such as lipopolysaccharide (LPS) and fimbriae, play important roles in the induction of innate immune responses, including cytokine production by localized and circulating leukocytes, such as monocyte/macrophage. Based on our preliminary data, we hypothesize that live P.g. stimulates unique pro-inflammatory signal transduction pathways in human PBM as opposed to purified bacterial components, such as P.g. LPS or P.g. fimbriac. To test this, the following specific aims are proposed: Aim 1: To test at the transcriptional level the hypothesis that unique signaling pathways are differentially induced by live P.g., P.g. LPS and P.g. fimbriae in human peripheral blood monocytes. Aim 2: To test at the level of protein expression the hypothesis that unique signaling pathways are differentially induced by live P.g., P.g. LPS and P.g. fimbriac in human PBM. Aim 3: To establish the functional significance of unique signaling pathways identified in Aims 1 and 2 we will test them in vitro and in vivo. The results of these studies should provide a new gateway to our understanding of the etiology and pathogenesis of periodontal diseases. Since the differential host innate response identified in our preliminary data may in fact represent different stages of the periodontal infection, the results are expected to have important therapeutic implications.

**Grant:** 1R01DE016125-01  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** GRIFFEN, ANN L. MS MOLECULAR GENETICS  
**Title:** Molecular Analysis of the Biofilm in Caries and Health  
**Institution:** OHIO STATE UNIVERSITY COLUMBUS, OH  
**Project Period:** 2004/08/01-2009/04/30

**DESCRIPTION:** Dental caries is the most common chronic disease of childhood, and is the biggest unmet health care need among America's children. Socioeconomic disparities in both rates of disease and treatment are a major public health issue. To date, effective biological interventions to prevent caries have not been developed. Dental plaque contains several hundred different organisms, many of which are poorly studied. A number of species have been shown to produce sufficient acid to drive pH below critical levels and to tolerate low pH. Research has primarily focused on *Streptococcus mutans* as the etiologic agent in caries, but based the work of previous investigators using cultivation and on our preliminary findings using molecular methods, *S. mutans* is not always present in caries, is often found at low levels, and additional and unexpected bacterial species may be important. In addition, comparatively little attention has been paid to identifying health-associated and potentially beneficial bacterial species that may reside in the oral cavity. For the proposed project, bacterial species present in childhood caries and health will be identified by cloning and sequencing bacterial 16S ribosomal genes amplified from DNA isolated from plaque samples. This open ended approach will allow the detection and identification of all bacterial species present, including novel, uncultivated or unexpected species. The presence and quantities of the species identified by this approach as potentially associated with caries or health will then be determined using quantitative, real-time PCR. This dual-technique approach will allow the examination of a much larger sample size than is possible by cloning and sequencing alone. Accomplishment of the proposed studies will identify the pathogens associated with the onset and progression of severe caries of the primary and young permanent dentition, and identify those bacterial species associated with a healthy dentition. This study will also provide a comprehensive catalog of the supragingival flora in children based on molecular technology. The significance of the proposed work is that identification of additional caries pathogens would provide alternative targets for biological interventions, and identification of beneficial health-associated species could provide the basis for therapeutic interventions to establish caries-resistant microbial communities.

**Grant:** 1R01DE016133-01  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** KACHLANY, SCOTT C PHD  
**Title:** Leukotoxin production by *A. actinomycetemcomitans*  
**Institution:** UNIV OF MED/DENT NJ NEWARK NEWARK, NJ  
**Project Period:** 2004/07/01-2009/03/31

**DESCRIPTION:** *Actinobacillus actinomycetemcomitans* is a bacterium that is the etiologic agent for localized aggressive periodontitis (LAP). LAP is a destructive and aggressive disease of the oral cavity that affects adolescents. The incidence of LAP varies among population groups, but afflicts minorities and the underprivileged at a higher frequency. Failure to treat LAP results in the loss of teeth and other health-related problems. In addition, *A. actinomycetemcomitans* is part of the HACEK group of bacteria that causes infective endocarditis, a disease of heart valves and tissue. *A. actinomycetemcomitans* secretes a protein toxin known as leukotoxin. Leukotoxin destroys leukocytes of humans, and likely plays a significant role in the pathogenesis of *A. actinomycetemcomitans* by helping the bacterium evade the immune response. Leukotoxin is an RTX (repeats in toxin) toxin that includes other important toxins such as *E. coli* alpha-hemolysin, *M. haemolytica* leukotoxin, *B. pertussis* adenylate cyclase, and *V. cholerae* RTX toxin. To date, little is known about how *A. actinomycetemcomitans* leukotoxin is produced, activated, and secreted from bacterial cells. In addition, none of the RTX toxins have been crystallized to have their three-dimensional structures solved. Proposed here are experiments that will (1) identify the genes that are required for production of active leukotoxin, (2) study the genes and proteins using genetic and biochemical approaches, and (3) grow crystals of leukotoxin and solve its three-dimensional structure at the atomic level. We expect this work to lead to a better understanding of leukotoxin production and how the toxin contributes to disease. The structural information gained through these experiments will shed more light on the mechanism of action of this important class of toxins. This new information may lead to the design of therapeutic agents that can disrupt leukotoxin activity and ultimately treat or prevent disease.

**Grant:** 1R03DE015706-01A1  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** LI, YIHONG MD CLINICAL DENTAL  
SCIENCES, OTHER  
**Title:** Molecular epidemiological approach to determine S.mutans  
**Institution:** NEW YORK UNIVERSITY NEW YORK, NY  
**Project Period:** 2004/07/01-2006/04/30

DESCRIPTION (provided by applicant): Dental caries is the single most common chronic childhood disease. Each year, more than 51 million school hours are lost due to dental-related illness and over \$40 billion is spent on the treatment of this disease. Epidemiological and clinical studies have suggested that mutans streptococci, particularly *Streptococcus mutans*, are the major microbial pathogens associated with dental caries. The most commonly used technique to identify *S. mutans* is cultivation on selective media. The major limitations of the method include inadequate detection of *S. mutans* in saliva particularly when *S. mutans* is present at low levels; morphology varies depending upon the medium used; and it is costly and labor-intensive. To date, the most reliable technique to rapidly and specifically identify bacterial species is PCR. But for *S. mutans*, the lack of species-specific probes and primers continues to limit high-throughput research on prevalence and colonization of *S. mutans*. Therefore, the objective of this project is to develop highly sensitive and species-specific probes and primers that can be used in PCR-based assays to rapidly, accurately, and effectively identify *S. mutans* in clinical oral specimens. Our goal will be accomplished by pursuing the following specific aims. (1) To identify potentially unique sequences in *S. mutans* genome that will enable us to develop species-specific probes and primers for the detection of *S. mutans* in the clinical specimens. (2) To validate the probes and to demonstrate the high sensitivity and specificity of the species-specific probes and primers. (3) To compare the species-specific probes and primers with the conventional culture method. From these experiments, we should be able to obtain well-defined *S. mutans*-specific probes and primers and to prove the superiority of the newly developed probes to the findings obtained from the culture method. The new molecular markers will enable us to conduct molecular epidemiological studies of *S. mutans* infection and high-throughput research so that we can improve our understanding of the polymicrobial etiology of dental caries, ascertain a child's risk potential prior to disease development, and evaluate the effectiveness of caries interventions. Application of the new molecular tools will have a substantial impact on improving the oral health of children.



**Grant:** 1R21DE015564-01A1  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** BOWEN, WILLIAM H  
**Title:** Glucosyltransferase as a Marker for Caries Activity  
**Institution:** UNIVERSITY OF ROCHESTER ROCHESTER, NY  
**Project Period:** 2004/07/01-2006/05/31

**DESCRIPTION:** Dental caries involves the interaction of host molecules, bacterial components, dietary substrate and time. Tests for assessing caries activity, and the likelihood of developing new lesions include determination of level of infection by using salivary mutans streptococci and lactobacilli counts [Rogosa, et al., 1951; Duchin and van Houte 1978; Beighton, 1991], determination of aciduric organisms in plaque and saliva [Snyder, 1951; Grainger et al., 1965], and assessment of prior caries activity [Grainger and Nikiforuk, 1960; Stamm, 1993; Hausen, 1997; Powell, 1998; Messer, 2000]. There is a need for a simple, reliable and reproducible test to determine caries in affected individuals, all of which have shortcomings. Glucosyltransferases (Gff, E.C. 2.4.1.5) are the only proven virulence factors in the etiology of Dental caries identified from *Streptococcus mutans* thus far [DeStoppelaar et al., 1971; Hamada et al., 1984; Tanzer et al., 1985; Yamashita et al., 1993]. Levels of active Gff in saliva correlate with salivary populations of *S. mutans* [R611a et al., 1983; Scheie et al., 1987; Vacca-Smith et al., 1996b]. Given that Gffs are proven virulence factors in the pathogenesis of Dental caries and are present in whole saliva, it appears prudent to explore the development of a test using the enzyme(s) as a marker(s) for caries. To this end we will test saliva from either caries free children, or children with Early Childhood Caries, for Gff (contributed by both mutans and non-mutans streptococci) by direct enzyme assay. We will also determine the quantity(ies) of GffB, GtfC, and GtID of *S. mutans* in the subjects' saliva using monoclonal antibodies in an enzyme-linked immunosorbent assay. We will then attempt to correlate the assayed activity of Gff (from both mutans and non-mutan streptococci) with the concentrations of GtfB, GffC and GffD of *S. mutans*. Finally, we will determine whether we can correlate both the concentrations of GtfB, GtfC, and GffD, and the overall assayed Gff activity in saliva, with the current levels of clinical caries experience of the subjects. If successful in showing, in this first-step cross-sectional pilot study, a correlation between overall Gff activity, or level of individual Gffs in saliva, with caries prevalence, then the way might be open to explore in the future, in a second, longitudinal study, (separate from this application) whether Gtf in saliva can be used as a predictor of future caries development. Our eventual goal (in a future application) is to determine caries risk well before the onset of lesions and to identify those who are caries active short of cavitation or even white spot formation.

**Grant:** 1R21DE015571-01  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** LEE, SEOK-WOO DDS  
**Title:** Proteomics study of *Bacteroides forsythus*  
**Institution:** COLUMBIA UNIVERSITY HEALTH SCIENCES NEW YORK, NY  
**Project Period:** 2004/05/01-2006/02/28

**DESCRIPTION:** Pathogenic microorganisms survive and cause damage within the host by expressing specific gene products called virulence factors. Despite emerging evidences indicating that *Bacteroides forsythus* (Bf) is a crucial periodontopathic bacterium, the exact mechanisms whereby this microorganism initiates and mediates the pathogenic process are virtually unknown because of the limited information on its virulence factors. Therefore, in order to advance understanding of Bf infection, it is required to identify and characterize unknown virulence factors of Bf. Preliminary data presented in this application indicated that comparative 2-dimensional gel electrophoresis (2-DE) could be used to detect differentially expressed proteins of Bf, and the proteins selectively expressed in vivo were thus discovered. The purpose of this application is to identify and characterize in vivo-expressed proteins of Bf using proteomics study. It is expected that this novel approach, combined with the analysis of the Bf genome sequence, will lead to detection, identification, and characterization of potential virulence factors of Bf. In order to achieve this goal, the following Specific Aims are proposed: 1) to detect differentially expressed proteins of Bf by 2-DE. By comparing the protein profiles of in vitro- and in vivo-grown Bf cells using 2-DE, the proteins selectively expressed in vivo will be detected; 2) to Identify and characterize differentially expressed proteins using mass spectrometry and database search. Identity of in vivo-expressed proteins will be determined by mass spectrometry and search for Bf genome and protein database. This approach will provide a foundation for generating novel hypotheses that can be tested in future studies, in which authentic virulence function of the candidate virulence factors can be validated. The results obtained from these studies will assist to achieve a long-term goal of this laboratory: to gain a better understanding of the pathogenic of Bf by providing more information on its virulence factors.

**Grant:** 1R21DE015656-01  
**Program Director:** MANGAN, DENNIS F.  
**Principal Investigator:** LI, LING PHD  
**Title:** Vascular Tissue Invasion by *Porphyromonas gingivalis*  
**Institution:** NOVA SOUTHEASTERN UNIVERSITY FT LAUDERDALE, FL  
**Project Period:** 2004/01/01-2005/11/30

**DESCRIPTION:** *Porphyromonas gingivalis* (P. g.), a Gram-negative anaerobe is known to play a critical role in the development of periodontitis. Several lines of evidence had also indicated its role in the development of cardiovascular disease (CVD). First, epidemiological studies have concluded that individuals with periodontitis were at greater risk for CVD. Second, 16S rDNA of P. g. was found to be present in atheromatous plaques. In vitro, P. g. has been shown to invade human coronary artery endothelial cells (HCAEC) and coronary artery smooth muscle cells (CASM). In vivo, the long-term systemic challenge of Pg can accelerate atherogenic plaque progression in apolipoprotein E-deficient mice. Recently, we have found the presence of live bacteria in atherosclerotic tissue from patients. All these observations suggest that P. g. may invade cardiovascular tissues, thus contributing to the development of atherosclerosis by interfering with cellular function of host tissues and by eliciting an inflammatory response. Therefore, the central hypothesis is that P. g. contributes to the development of atherosclerosis by invasion of tunica intima and intima media. The Specific Aims of this study are to probe the ability and mechanism of cardiovascular tissue invasion and penetration by P.g. using novel human organ and cell culture systems. Human saphenous vein organ culture that maintains 3-D structure of vascular tissue will be used for the first time to evaluate invasion and penetration ability of P.g. The depth and amount of invaded P.g in tunica intima and intima media will be determined by real time quantitative PCR and immunofluorescent microscopy using antibodies against P.g., endothelial and smooth muscle cell markers. By this system, we will 1) determine whether invasion can lead to neointima formation. 2) compare the invasion ability in injured and non-injured veins. 3) determine the relationship of demographic and medical data of vein donors with the susceptibility of the invasion. To probe the transcellular or/and paracellular route(s) of tissue penetration by P.g., primary endothelial and smooth muscle cell lines will be co-cultured in a mono-layer or bi-layer transwell culture system in which endothelial cells will be kept in transwell inserts, resembling in vivo layout. The ability of P.g. to either exit the pre-infected endothelial cell layer or penetrate the un-infected endothelial cell layer to invade smooth muscle cells will be evaluated. In parallel, the endothelial cell layer morphology and integrity will be monitored by scanning electronic microscope and the status of endothelial cell junctions will be detected by measuring transendothelial resistance. The proposed experiments will determine whether P.g can invade and penetration vascular tissue through transcellular or/and paracellular pathway(s). The completion of this study will contribute to the long-term goal of understanding this bacterially induced vascular inflammation and identification of new therapeutic targets.

**Grant:** 1R21DE015786-01  
**Program Director:** BHARGAVA, SANGEETA  
**Principal Investigator:** TENG, YEN-TUNG A PHD ORAL PATHOLOGY  
**Title:** Therapeutic scFv antibody for human periodontitis  
**Institution:** UNIVERSITY OF ROCHESTER ROCHESTER, NY  
**Project Period:** 2004/04/01-2006/03/31

**DESCRIPTION:** Human periodontal (gum) disease (i.e., gingivitis, and periodontitis - a more severe form of gingivitis) is the result of infections associated with specific oral bacteria below the gum lines. Chronic inflammatory periodontitis is the leading cause of tooth loss in adults (prevalence, >80 percent worldwide). Approximately \$15 billion dollars per year are spent on its diagnosis, professional cleaning and treatment including the replacement of affected teeth in the United States. Recently, severe periodontal disease has been shown to be associated with an increased risk for coronary heart disease, stroke, bacterial pneumonia, diabetes and undesired pregnant outcomes. Conventional periodontal treatment relies on mechanical debridement of the affected teeth and gums by scaling, root planning and surgeries, sometimes combined with antibiotic usage, which suffers from nonspecific removal of the target microorganisms, high costs, time consuming, emergence of antibiotic resistance strains, re-infection of the gums etc. Therefore, human periodontal disease is a significant health and healthcare issue. In the current application, the applicant proposed to develop a new generation of neutralizing human single-chain Fv monoclonal antibodies (called: scFv-MoAbs) against a recently defined critical microbial target involved in human periodontitis, CagE homologue: a death-inducing protein of an etiological human periodontal pathogen, *Actinobacillus actinomycetemcomitans*, from infected gum tissues. Importantly, the resulting neutralizing monoclonal antibodies will be tested in a "humanized" mouse model established by the applicant to validate the efficacy of the scFv-MoAbs being assessed before approaching human clinical studies. This combined new technology and specifically targeted approach by applying scFv-MoAbs to modulate or treat periodontal infections, if successful, will likely to become the new therapy for gum diseases and infections in the 21st century. Therefore, the patients' periodontal health will be improved and, eventually, this new treatment modality will also alleviate the chronic or/and long-term exposure of periodontal infections to systemic complications or risks; thereby, reducing the health-care burden and the associated socio-economical costs.

**Grant:** 1R21DE015972-01

**Program Director:** BHARGAVA, SANGEETA

**Principal Investigator:** DALE-CRUNK, BEVERLY A PHD  
BIOCHEMISTRY:BIOCHEMISTR  
Y-UNSPEC

**Title:** Protease-activated receptor signaling in oral innate im\*

**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA

**Project Period:** 2004/04/01-2006/03/31

**DESCRIPTION** (provided by applicant): This exploratory proposal will utilize genomic techniques to analyze the molecular interactions that occur between oral bacteria and host epithelial cells associated with the pathogenesis of periodontal disease. Studies will focus on the role of the proteinase-activated receptor (PAR) family in signaling innate immune and inflammatory responses by gingival epithelial cells. Members of this family of receptors are expressed on both gingival epithelial cells and fibroblasts. These receptors signal the presence of danger in the environment, contribute to inflammation and to enhance epithelial cell phagocytosis. However, their role in periodontal disease is poorly understood. Multiple factors produced during the chronic inflammatory response appear to be major factors in the tissue destruction that occurs in periodontal disease. The major microbial species associated with periodontal disease all have proteinases as part of their set of virulence factors. The studies of this proposal will lay the groundwork for further studies on the role of PARs, define a possible new molecular mechanism for pathogenesis of disease, and open the way to understanding new therapeutic targets to prevent or treat periodontal diseases. This work will test the hypotheses (1) that PARs are involved in innate immune function in gingival epithelium and (2) that signaling via PAR-1 and PAR-2 (the most highly expressed members of this family) differentially contributes to the innate immune response in these cells thereby activating different networks of response genes. This work will also examine the possible role of PARs in the recognition of both commensal and pathogenic oral bacteria by gingival epithelial cells. The aims of the proposal are (1) to characterize the involvement of PAR signaling in upregulation of human beta-defensin-2 and other markers of innate immune activation in oral epithelial cells using the PAR-1 activator, thrombin, and PAR-2 peptide agonist peptide, SLIGRL-NH<sub>2</sub>, and oral pathogenic and commensal bacteria and (2) to utilize gene array analysis to test the hypothesis that PAR-1 and PAR-2 signaling differentially contributes to the innate immune response in gingival epithelial cells to activate different networks of response genes.

**Grant:** 1R21DE015980-01  
**Program Director:** BHARGAVA, SANGEETA  
**Principal Investigator:** BARBOUR, SUZANNE E PHD MOLECULAR  
BIOLOGY/GENETICS  
**Title:** Monocyte Modulation in LAgP  
**Institution:** VIRGINIA COMMONWEALTH UNIVERSITY RICHMOND, VA  
**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (provided by applicant): Localized Aggressive Periodontitis (LAgP) is an inflammatory disorder of the periodontal tissues that primarily affects molars and incisors. LAgP is an infectious disease and has been linked to several oral pathogens, most notably *Actinobacillus actinomycetemcomitans* (Aa) and *Porphyromonas gingivatis* (Pg). However, the host response contributes to disease progression and may dictate the pattern and severity of the disease. Several lines of evidence suggest that LAgP monocytes exhibit a unique phenotype. For example, these cells exhibit exaggerated responses to gram negative LPS, reduced catabolism of the bioactive lipid platelet-activating factor (PAF), and produce soluble mediators that enhance the IgG2 antibody response, while non-periodontitis (NP) subjects monocytes do not have these effects. Although both LAgP and NP monocytes differentiate into monocyte-derived dendritic cells (DC), a higher percentage of LAgP monocytes undergo this process, suggesting that monocyte differentiation in LAgP is skewed to the DC phenotype. Based on these observations, we hypothesized that the gene expression profiles of LAgP and NP monocytes would differ and used microarray analyses to address this question. Our preliminary studies indicate that >600 genes are differentially expressed in the two groups of monocytes. Many of these are associated with innate and adaptive immunity, including genes involved in Th1/Th2 responses, chemotaxis, antigen presentation, and interferon signaling. Thus, these initial microarray experiments have provided us with several "leads" that will help in the further characterization of LAgP monocytes, their roles in the immune response against oral pathogens, and how their biology might both promote immunity and permit these chronic infections to persist. We now propose to use more quantitative methods to accurately compare gene expression in LAgP and NP monocytes. In addition, biological assays will be performed to assess the functional consequences of the changes in gene expression. It is possible that the LAgP monocyte phenotype is strictly related to the genotype of LAgP subjects. However, oral bacteria can modulate monocyte biology, suggesting that interactions between LAgP monocytes and Aa or Pg could generate the changes in gene expression. To address this issue, NP monocytes will be cultured together Aa or Pg and the microarray approach will be used to compare gene expression profiles in these cells to NP monocytes that have not been exposed to bacteria. Together, these studies should provide us with a clearer understanding of host-pathogen interactions in LAgP and potentially with mechanisms for modifying these interactions and thereby controlling this chronic infectious disease.

**Grant:** 1R21DE016313-01  
**Program Director:** HUNZIKER, ROSEMARIE  
**Principal Investigator:** QI, FENGXIA PHD  
**Title:** Piezoelectric QCM for Dental Caries Research  
**Institution:** UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA  
**Project Period:** 2004/09/28-2006/06/30

**DESCRIPTION:** Piezoelectric quartz crystal microbalance (QCM) is a powerful tool to measure mass accretion on surfaces in the nanogram range. In the past 10 years, it has found more and more applications in a variety of biological studies from protein-protein interaction, DNA hybridization, surface attachment and biofilm formation, to detection of pathogens in food, water, and urine samples. Nevertheless, this powerful technique has not been applied in Dentistry. In this exploratory research application, investigators from UCLA School of Dentistry (UCLA) and Chemical and Environmental Engineering Department of University of California-Riverside (UCR) are partnering to introduce this technology to Dentistry by developing an integrated system combining QCM with fluidic chamber adaptable to laser scanning confocal microscopy (LSCM). We posit that this integrated system will allow for real-time measurement of biomass accretion with simultaneous analysis of structural development during biofilm formation. It will also allow for rapid, sensitive, and specific detection of oral pathogens with simultaneous image confirmation. Two Specific Aims are proposed for this study. Aim 1 is to develop a LSCM compatible, integrated QCM/fluidic chamber system for oral biofilm studies. Aim 2 is to apply QCM for rapid detection and quantification of *S. mutans* in mixed cultures. The outcome of this study will be the novel development and application of the QCM technology in Dentistry, which will result in acquisition of new knowledge in Dental biofilm research and development of a novel diagnostic tool that has the potential to be developed into a chair side device for oral pathogen detection.

**Grant:** 1R21DE016404-01  
**Program Director:** BHARGAVA, SANGEETA  
**Principal Investigator:** HALE, LAURA P  
**Title:** Novel Oral Adjuvant for Dental Vaccines  
**Institution:** DUKE UNIVERSITY DURHAM, NC  
**Project Period:** 2004/09/28-2006/08/31

**DESCRIPTION:** Bacterial infections of the teeth and gingiva commonly cause tooth decay and periodontal disease, but may also cause serious systemic diseases such as endocarditis. Vaccines have a demonstrated potential to prevent infectious diseases. Co-administration of antigen with an immune stimulant called an "adjuvant" is usually necessary to stimulate the development of protective immunity rather than tolerance, particularly in response to oral antigens. Bromelain is a natural mixture of proteinases derived from pineapple stem. Although ingestion of most proteins results in immune tolerance, our preliminary data show that bromelain generates strong systemic immune responses when administered orally. Thus bromelain may serve as a self-adjuvant for induction of anti-bromelain antibodies. Our data show that bromelain is inhibited by trapping in complexes with the broad spectrum proteinase inhibitor alpha-2-macroglobulin (alpha-2M). Alpha-2M complexes are efficiently taken up by receptors that are present on antigen-presenting cells. We posit that proteolytically active bromelain given orally interacts with alpha-2M in vivo to form complexes that efficiently induce immune responses. Based upon this hypothesis, antigens co-trapped with bromelain in alpha-2M complexes should also be strongly immunogenic. The Aim of this study is to investigate the role of bromelain proteolytic activity in induction of antibody responses against itself and co-administered antigens, using an oral vaccine against Dental pathogens in mice. Bromelain proteolytic activity will be increased by formulation in antacid or permanently inactivated by reduction and alkylation. Bromelain will be administered orally to mice mixed with or covalently linked to antigen. Specific serum IgG and salivary IgG and IgA antibody responses against bromelain and antigen will be determined. If these studies confirm the potential of bromelain as an adjuvant, preparations containing bromelain and a mixture of relevant antigens could be developed for periodic administration as "swish and swallow" vaccines to induce and maintain immunity against Dental pathogens.



**Grant:** 1R21DE016474-01  
**Program Director:** CANTO, MARIA TERESA  
**Principal Investigator:** FINE, DANIEL H DMD CLINICAL  
DENT:PERIODONTIA  
**Title:** Microbial Markers for Periodontal Disease in Children  
**Institution:** UNIV OF MED/DENT NJ NEWARK NEWARK, NJ  
**Project Period:** 2004/09/10-2006/06/30

**DESCRIPTION** (provided by applicant): The primary objective of this pilot study is to determine whether the presence of *Actinobacillus actinomycetemcomitans* (Aa), a "Virulent" clone of Aa (the JP2 clone), or specific microbial complexes could be predictive of the initiation and progression of Localized Aggressive Periodontitis (LAP) in periodontally healthy African-American and Hispanic children, two populations with a high prevalence of LAP. The first aim will be to investigate the relationship between the presence of Aa in healthy children and subsequent disease initiation. The second aim will be to determine whether the presence of specific virulent clones of Aa will initiate conversion from health to disease in those children. The third aim will be to determine whether specific pathogenic microbial complexes are associated with disease in the two study populations (crosssectional study) and, if so, whether these "pathogenic complexes" are related to disease initiation (prospective study). 1,200 students, ages 11-14, from Newark will receive a baseline periodontal exam. Bacteria will be collected from each student for identification of Aa by cultural methods and 40 other plaque bacteria by DNA/DNA checkerboard hybridization. 240 Aa culture positive periodontally healthy students and 240 Aa negative matched controls will be selected for the prospective study and examined and sampled every 6 months for two-years. Disease initiation will be detected by increased probing attachment levels and radiographic evidence of bone loss. Statistical analysis will determine whether there is a relationship between students who harbor the target microorganisms (Aa, clones of Aa, or specific microbial complexes) and disease initiation and will be used to determine the design of the proposed follow-up clinical trial: The long-term goal of this proposal is to use the pilot data generated to plan for a definitive clinical trial intended to design strategies for early identification of children at risk for LAP so that cost effective preventive interventions can be developed to significantly reduce the burden of disease in this underserved population.

**Grant:** 1R21DE016499-01  
**Program Director:** CANTO, MARIA TERESA  
**Principal Investigator:** PETERS, MATHILDE DMD  
**Title:** Microbial Outcomes of Minimal Intervention Treatment  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2004/09/01-2006/06/30

DESCRIPTION (provided by applicant): This study proposes to deliver microbiological evidence that complete removal of carious dentin from human teeth is not necessary in order to prevent further progression of the disease, as long as the margins of a restoration are sealed against the ingress of bacterial nutrients. The concept of incomplete caries removal is not new, and has been convincingly supported by studies of Handelman, Mertz-Fairhurst and others. Nowadays, indirect pulp treatment is an accepted and advised option for treating deep carious lesions. In this procedure the carious dentin removal is left incomplete in order not to endanger pulpal health. One study also reports that carious dentin was left in 72% of cavities which were deemed to be caries free (Anderson '85). It is improbable to suggest that all these teeth then became non-vital! There is also indirect evidence from the literature to support this concept. However, it has not yet become the treatment of choice in general dental practice, although such treatment would certainly conserve more tooth tissue; be less traumatic for the patient; less time consuming for dentist and patient; perhaps less expensive; and be a treatment option in communities where access to care is problematic because of a lack of trained dental personnel. Biologically there are also several important questions to be answered. Current molecular techniques may provide definitive answers to the reduction in numbers and viability of the "entombed" microflora. This study aims to sample carious dentin (n=30) after incomplete caries removal at baseline and at 6 months after sealing, to investigate the residual bacteria, i.e. total bacterial load, distribution of various species and metabolic activity of the microbiota involved. Qualitative clinical and radiographic data will be complemented by current molecular techniques. Quantitative PCR and real-time RT-PCR will allow for quantitative analysis and reveal a more complete picture of the persistence of cariogenic bacteria. This investigation is a clinical and molecular re-entry study of sealing of caries, in support of a future full-scale clinical trial of incomplete caries removal.

**Grant:** 2R01DK013332-35  
**Program Director:** SECHI, SALVATORE  
**Principal Investigator:** FRIEDEN, CARL PHD CHEMISTRY:CHEMISTRY-UNSPEC  
**Title:** Intermediates in Protein Folding  
**Institution:** WASHINGTON UNIVERSITY ST. LOUIS, MO  
**Project Period:** 1977/01/01-2009/06/30

DESCRIPTION (provided by applicant): The long-term goal of this proposal is directed at the experimental determination of the mechanism(s) of protein folding using NMR methods. For these studies we incorporate specific fluorine labeled amino acids into proteins and use one-dimensional NMR to examine the rates of side chain stabilization during folding as well as the appearance and disappearance of folding intermediates. These rates are important because they may define rates of macromolecular interactions, ligand binding and posttranslational modifications in vivo. We plan to investigate, analyze and interpret the kinetics of side chain packing and stabilization during protein folding. Proteins currently under study include the E. coli dihydrofolate reductase, the intestinal fatty acid binding protein, PapD, a chaperone for pilus formation in pathogenic bacteria and adenosine deaminase. Misfolded proteins have now been implicated in a number of neurological diseases, particularly Alzheimer's. In order to understand misfolding, we must understand the mechanism of folding itself. The lack of adenosine deaminase activity as a consequence of mutations distant from the active site, for example, leads to Severe Combined Immunodeficiency Disease in children. Experiments with wild type and mutant CuZn superoxide dismutase are also planned. Mutants of this enzyme are known to cause familial amyotrophic lateral sclerosis (fALS). A second project is to investigate proline isomerization during folding by directly measuring the rate of cis/trans isomerization of every proline in proteins containing multiple proline residues after incorporating 3-<sup>19</sup>F-proline. Both these projects employ novel real-time and equilibrium NMR methodology in addition to standard.

**Grant:** 2R01DK042648-14  
**Program Director:** NYBERG, LEROY M.  
**Principal Investigator:** SCHAEFFER, ANTHONY J  
**Title:** INDUCTION AND MODULATION OF HOST RESPONSES BY UPEC  
**Institution:** NORTHWESTERN UNIVERSITY EVANSTON, IL  
**Project Period:** 1990/04/01-2007/11/30

DESCRIPTION (provided by applicant): Urinary tract infections (UTIs) cause significant morbidity and are a major burden on our healthcare system. Most UTIs are due to ascending infections by uropathogenic *E. coli* (UPEC) that leads to an inflammatory response in the bladder characterized by elevated urinary cytokines and apoptosis of superficial urothelial cells. We have previously observed that UPEC has a novel activity that suppresses activation of the inflammatory regulator NF $\kappa$ B, and we now demonstrate that clinical *E. coli* isolates and laboratory strains differentially induce multiple inflammatory genes and cytokines. Thus, our hypothesis is that UPEC encodes virulence factors that induce and modulate the host inflammatory response. In contrast to previous studies that examine one or a few strains in a single genetic background, this project will compare various *E. coli* strains that mediate acute disease as well as strains that induce benign conditions using urothelial cultures corresponding to normal patients and patients with benign conditions. By comparing *E. coli* isolates in a variety of host backgrounds in culture using ELISA to quantify cytokine secretion (Aim 1), we will establish the relative roles of pathogen and host in determining inflammatory responses. Aim 2 will define the diversity of genes that are modulated by UPEC and whether this modulatory activity is present in other *E. coli* strains of diverse clinical origin using real-time RTPCR to quantify inflammatory markers. In vivo analysis (Aim 3) using the mouse model will identify the role of specific cytokine responses in determining the course of infection for diverse *E. coli* strains by characterizing the timecourse of infection and severity of inflammation. Finally, using a genetic screen based on suppression of IL-8 expression, Aim 4 will identify the activity of UPEC that suppresses NF $\kappa$ B, provide initial characterization of its mechanism of action, and determine its conservation among *E. coli* strains of diverse clinical origin. This project will thus enhance our understanding of the roles of pathogen and host in infection and may lead to the identification of new therapeutic targets for the treatment and management of UTIs.

**Grant:** 2R01DK048373-08A1  
**Program Director:** HAMILTON, FRANK A.  
**Principal Investigator:** FASANO, ALESSIO MD  
**Title:** Tight junctions and role in diarrhea of zot  
**Institution:** UNIVERSITY OF MARYLAND BALT PROF BALTIMORE, MD  
SCHOOL  
**Project Period:** 1996/05/01-2007/04/30

**DESCRIPTION** (provided by applicant): The paracellular route is the dominant pathway for passive solute flow across the intestinal epithelial barrier, and its permeability depends on the regulation of the intercellular tight junction (tj), also known as the zonula occludens (ZO). The tj is a dynamic and complex structure whose physiological regulation remains largely undefined. During the 7 years of funding of this grant, our studies have focused on the mechanism(s) of action of a protein elaborated by *Vibrio cholerae*, zonula occludens toxin (Zot). Our experiments using Zot as a tool to gain insights into the regulation of tj function led to the discovery of zonulin, a human eukaryotic Zot analogue, and to the definition of some of its physiological (innate immune mucosal response of the gut) and pathological (autoimmune diseases) roles. We have demonstrated that Zot and zonulin each target the same surface intestinal receptor and activate the same intracellular signaling events that lead to reversible tj disassembly. We have extended our findings to disease states characterized by a leaky gut and have established the role of zonulin in their pathogenesis. We have applied genetic, biochemical, and physiologic techniques to define the structural and functional requirements for activation of the zonulin system. Our overall hypothesis is that the 12 kDa N-terminal portion of Zot and its mammalian analogue zonulin target the same specific receptor (the protease activated receptor (PAR)2) preferentially expressed on the surface of mature cells of the small intestine. This receptor-ligand interaction is coupled to activation of phospholipase C (PLC), protein kinase C (PKC) $\alpha$ , actin polymerization, and tj disassembly. We hypothesize that PKC-mediated phosphorylation of tj proteins alter protein-protein interaction within the tj multiprotein complex leading to the reversible tj disassembly. The long-term objective of this proposal is to use Zot as a probe to elucidate the role of the zonulin system in physiological and pathological states. We will focus on the structural requirements to activate the intracellular signaling involved in Zot/zonulin-mediated tj disassembly, and on establishing the Zot/zonulin-mediated activation of PAR-2 to gain insights into tj regulation at the cellular and molecular levels in health and disease.

**Grant:** 2R01DK053708-06A2  
**Program Director:** HAMILTON, FRANK A.  
**Principal Investigator:** GOLD, BENJAMIN D MD  
**Title:** Risk factors for gastric disease in pediatric H. pylori  
**Institution:** EMORY UNIVERSITY ATLANTA, GA  
**Project Period:** 1997/09/30-2007/05/31

DESCRIPTION (provided by applicant): *Helicobacter pylori* (Hp) is a major cause of chronic-active gastritis, primary duodenal ulcers and strongly linked to gastric cancer. Most Hp infections worldwide are acquired in childhood. Why some individuals develop symptomatic disease is unclear and, until recently, no studies critically evaluated the role of pediatric Hp strains and/or host factors in disease outcomes. Over the past 5 years of NIH funding, 486 children from Atlanta, Cleveland, and Miami were enrolled; 184 (38%) were Hp-infected. Race (African American) and younger age, in conjunction with Hp strains expressing *cagA* and *vacAs1 B*, were shown to be risk factors for both esophageal and gastric disease; suggesting a different disease paradigm from Hp-infected adults. Using the Updated Sydney system, we demonstrated a histopathologic spectrum in children, which included novel observations of atrophic gastritis with intestinal metaplasia. Overall hypothesis for competitive renewal: disease manifestations in Hp-infected children are influenced by specific host factors (i.e., race, immune phenotype), environmental exposures, and specific virulence factors of infecting Hp strains. Specific aims: 1) Using well defined cases and controls, further characterize specific host factors and environmental exposures contributing to symptomatic childhood infection emphasizing targeted enrollment in specific age, gender and demographic strata to facilitate detection of significant differences not attained previously and follow up of 2 established specific cohorts to ascertain immune response natural history. 2) Utilize gene-array technology for whole Hp genome assessment and bacterial gene expression of specific virulence determinants associated with pediatric Hp strains. 3) Further, characterize the host immunologic and mucosal response in Hp infected children. Hp-infected symptomatic endoscopy cases at our established three clinical centers of high, moderate and low tip prevalence will be compared with age-matched Hp-infected asymptomatic and uninfected symptomatic controls. Two geographically and demographically distinct centers have been added to provide additional geographic and subject representativeness to the patient cohort. The Updated Sydney system will be employed to assess gastric histopathology severity and phenotype in newly enrolled cases in specific age, gender and demographic strata and follow-up of the two "novel" cohorts established in the past 5 years; a) atrophic gastritis; b) esophageal and gastric disease group enabling a comprehensive, multivariate evaluation of the natural history of Hp-infected children in two distinct disease paradigms. Using molecular methods (multiplex [MP]-PCR, RT-PCR) and a micro ELISPOT assay on peripheral blood mononuclear cells (PBMCS), Th1, Th2, Th3 or balanced Th1/Th2 response will be determined to further characterize the Hp-infected child's immune response phenotype. We propose to further our previous work with critically lacking studies from a multivariate approach leading to a better understanding of the gastroduodenal disease sequelae and overall pathobiology of Hp infection in humans.

**Grant:** 1R01DK062185-01A1  
**Program Director:** HAMILTON, FRANK A.  
**Principal Investigator:** SEPULVEDA, ANTONIA R MD  
**Title:** H.pylori Effects on DNA Repair in Gastric Epithelium  
**Institution:** UNIVERSITY OF PITTSBURGH AT PITTSBURGH PITTSBURGH, PA  
**Project Period:** 2004/02/01-2008/12/31

**DESCRIPTION** (provided by applicant): The molecular mechanisms by which *H. pylori* (Hp) increases gastric cancer (GC) risk are vastly unknown. Direct interaction of Hp organisms in co-culture with gastric epithelial cells causes a marked decrease in the levels of the main DNA mismatch repair (MMR) proteins MLH1 and MSH2 and microsatellite instability (MSI)-type mutations in a reporter gene. Up to 30% of GC show MSI-High, gastric mucosa with chronic gastritis and intestinal metaplasia frequently show MSI-mutations, and patients with MSI-positive GC are more likely to have active Hp gastritis. These data lead to our hypothesis that Hp might cause mutation accumulation in the stomach epithelium by impairing DNA MMR, representing a pathway of GC development and explaining at least in part how Hp infection increases GC risk. Specific aim one: To determine the degree of MMR deficiency required for MSI mutation accumulation, to characterize the spectrum of mutational targets and mutations and to identify the fundamental mechanisms underlying the reduced levels of MLH1 and MSH2 proteins induced by Hp in cultured gastric epithelial cells (GEC). GEC and increasing numbers of Hp will be co-cultured. The levels of MLH1 and MSH2 proteins associated with MSI development will be determined. MSI mutation accumulation will be determined in GEC repeatedly exposed to Hp organisms using GFP reporter vectors by western and FACS analyses. We will determine the spectrum of mutations at polyCA and polyA repeats. Transcription rates, mRNA and protein stability of MSH2 and MLH 1 will be measured after Hp infection. Specific aim two: To characterize the alterations of MLH1 and MSH2 and level and frequency of MSI mutation accumulation during *H. pylori* gastritis in humans. Gastric epithelium will be obtained by laser capture microdissection using biopsies from Hp infected individuals before and after Hp eradication and from control non-infected patients. Protein and mRNA levels of MLH1 and MSH2 will be determined by western and Taqman analysis. Mutations in the epithelium of Hp infected individuals will be evaluated by examining a recommended panel of microsatellite markers and gene targets of MSI-mutagenesis. The MSI and MLH1 and MSH2 protein and mRNA levels will be evaluated before and after Hp eradication to test whether the changes induced by Hp are reversible. The long-term goals and impact of this study are to understand the Hp bacterial-host interaction mechanisms that lead to increased risk of GC. Characterization of MMR alterations and target gene mutations may become useful as a molecular tool for surveillance and GC risk assessment of patients with chronic Hp infection. Knowledge gained from this study is likely to support the indication for Hp eradication in *H. pylori*-infected patients to prevent GC.

**Grant:** 1R01DK062813-01A2  
**Program Director:** HAMILTON, FRANK A.  
**Principal Investigator:** YAMAOKA, YOSHIO MD  
**Title:** Regulation of CXC chemokine expression by *H. pylori*.  
**Institution:** BAYLOR COLLEGE OF MEDICINE HOUSTON, TX  
**Project Period:** 2004/06/01-2009/05/31

DESCRIPTION (provided by applicant): *Helicobacter pylori* causes gastric inflammation, peptic ulcer disease, and gastric cancer. Recent clinical and in vitro data indicate that both the cag pathogenicity island (PAI) and the outer inflammatory protein, OipA are involved in production of gastric mucosal interleukin (IL)-8 levels and the resulting inflammation. Published data aimed at understanding the cag PAI-associated IL-8 signal transduction pathway have provided inconsistent results possibly because the effect of OipA was not taken into account. This proposed study seeks to understand the IL-8 signal transduction pathway stimulated by *H. pylori* infection. I hypothesize that the oipA gene product activates the binding of interferon (IFN) regulatory factors (IRFs) to a novel interferon-stimulated responsive element (ISRE)-like element in the IL-8 promoter and that the oipA gene product activates p38 mitogen-activated protein (MAP) kinase cascades, which the cag PAI does not. My approach involves use of parental *H. pylori* strains and precise gene deleted mutants of oipA, cag PAI, cagA, hopZ or oipA/cag PAI genes without polar effects, as well as complemented oipA mutants. AIM 1 seeks to determine the mechanisms for regulation of IL-8 gene transcription in relation to OipA and cag PAI. I will test the hypothesis that OipA and/or cag PAI activates binding IRFs to the ISRE-like element, leading to IL-8 gene transcription. I will identify the MAP kinase pathways upstream of the IL-8 promoter related to OipA and the cag PAI. I will also determine whether there is cross-talk among the IRF-ISRE-like element pathway, the MAP kinase pathway, and IkappaB-NF-kappaB pathway upstream of the IL-8 promoter (i.e., are they linked or independent). Primary methods to be used include a) a luciferase reporter gene assay, b) electrophoretic mobility shift assays (EMSA) and supershift assay, c) microaffinity isolation assay, d) RNA interference, and e) western blot analysis. AIM 2 seeks to confirm and extend the in vitro results by investigation of the in vivo effect of OipA and the cag PAI on gastric injury using the Mongolian gerbil model. I will determine the effect of OipA and cag PAI on gastric inflammation, clinical outcome, CXC chemokine (KC and IP-10) induction and analyses of KC and IP-10 promoter related to OipA and the cag PAL. Primary methods to be used include a) real time reverse transcription-polymerase chain reaction, b) enzyme-linked immunosorbent assay, c) EMSA and supershift assay, and d) Western blot analysis. The results from these studies will provide new insights into the role of *H. pylori* in the pathogenesis of gastroduodenal disease.



**Grant:** 1R01DK062852-01A2  
**Program Director:** HAMILTON, FRANK A.  
**Principal Investigator:** MAIER, ROBERT J BS  
**Title:** Nickel Metabolism in Helicobacter pylori  
**Institution:** UNIVERSITY OF GEORGIA ATHENS, GA  
**Project Period:** 2004/03/15-2007/02/28

DESCRIPTION (provided by applicant): Helicobacter pylori is a spiral bacterium that colonizes the gastric mucosa of humans, leading to a variety of gastric diseases that include peptic ulcers, chronic gastritis, mucosal-associated lymphomas, and adenocarcinomas of the lower stomach and duodenum. Two nickel-containing enzymes, urease and hydrogenase, are important for the bacterium's mucosal-colonizing abilities. The goal proposed here is to understand the nickel sequestering, storage, metabolizing and metalloregulatory steps involved in the synthesis/maturation of the two nickel-containing enzymes. From studies with other organisms, the sequence-identified accessory proteins (encoded by ure and hyp genes) would be expected to form nickel sequestering and energy utilizing (GTP hydrolyzing) complexes needed for maturation of the two Ni-enzymes. In the presence of nickel, these complexes facilitate mobilization of the metal into the final sink, urease or hydrogenase. From our knowledge of purified nickel binding and GTPase accessory proteins and their interactions, a sequential Ni-mobilizing transfer pathway will be proposed. The hypothesized Ni-transfer steps will be tested by mixing together selected pure accessory proteins. The roles of some specific nickel-binding proteins (predicted from the genome sequence) in nickel sequestering, homeostasis, and regulation will be addressed via targeted mutagenesis. The specific approaches to understand nickel metabolism, homeostasis, and regulation, in H. pylori will include purification of complexes that deliver nickel to the Ni-enzymes, proteomic analysis to identify proteins, and genomic analysis of transcripts that are regulated in response to nickel supplementation. Finally, gene directed mutants in proteins that are anticipated to affect the nickel nutritional status of the bacterium (i.e. nickel homeostasis) will be characterized to understand the roles of three specific (but poorly studied) nickel binding proteins. The role of a DNA binding and nickel-sensing protein (NikR) is of particular interest as it may play a global regulatory role in metal homeostasis, in turn affecting many processes of cell metabolism.

**Grant:** 1R01DK064229-01A1  
**Program Director:** NYBERG, LEROY M.  
**Principal Investigator:** MOSELEY, STEPHEN L PHD  
**Title:** Structure, Function, and Evolution of Dr adhesins  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 2004/01/01-2007/12/31

DESCRIPTION (provided by applicant): The proposed research will study structure function relationships in the Dr family of adhesins. These adhesins are associated with diarrheal disease and urinary tract infections caused by E. coli. The adhesins are exceptional among E. coli adhesins in that they recognize protein receptors, rather than glycoconjugates. We will focus on one member of this family, the Dr hemagglutinin, and its adhesive subunit DraE, as a model. The receptors for this adhesin are the complement regulatory protein decay accelerating factor (DAF) and type IV collagen. We propose to characterize mutants we have isolated with altered DAF binding phenotypes for the ability to bind to the two known cell-associated form of DAF. We will carry out refined structural predictions which will form the basis of new mutagenesis experiments and deletion analysis of the adhesive subunit. We will study type IV collagen binding by the DraE adhesin, and test the hypothesis that the collagen binding domain is a conformational epitope comprised of amino acids from two neighboring subunits in the polymer. Common neutralizing epitopes of DraE which induce antibodies that inhibit the binding of other Dr adhesins will be sought. We will test the hypothesis that binding by the Dr hemagglutinin is enhanced by shear forces. The receptor domains of DAF and type IV collagen will be investigated by analysis of the ability of synthetic peptides to inhibit binding, and by crosslinking of the adhesin-receptor complex, followed by proteolysis and identification of crosslinked peptides by mass spectrometry. Strains will be constructed for the expression of chaperone-adhesive subunit complexes for crystallization studies. The evolution of divergence in the Dr family, a family characterized by exceptional variability considering the conservation of binding specificity, will be investigated to determine functional pathoadaptation. These studies will contribute to the understanding of diarrheal disease and urinary tract infections caused by E. coli, and will provide knowledge applicable to the development of new strategies for therapy and prevention.

**Grant:** 1R01DK064954-01A1  
**Program Director:** MCKEON, CATHERINE  
**Principal Investigator:** MILLER, SAMUEL I  
**Title:** P. aeruginosa as a surrogate marker of cystic fibrosis  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 2004/07/01-2007/05/31

DESCRIPTION (provided by applicant): Chronic pulmonary infection significantly limits the span and quality of life of children and young adults with cystic fibrosis (CF). CF is the result of mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene and individuals with CF have innate immune alterations, which in turn lead to chronic pulmonary infection, inflammation and, ultimately, airway destruction. The respiratory tracts of most patients with CF (>80% by age 15) are infected with the opportunistic gram-negative bacterium *Pseudomonas aeruginosa*, and such infection is clearly associated with poor outcome. The development of strategies to prevent *P. aeruginosa* colonization and eliminate chronic infection will require an understanding of the natural history of the bacterial component of CF. *P. aeruginosa* infection in CF involves colonization early in life. Over many years the bacteria adapt to the CF airway environment with an increased ability to replicate and stimulate inflammatory responses that are ineffective against the bacteria but damage airways. This grant proposes to define common characteristics of *P. aeruginosa* adaptation to the CF airway by analyzing bacteria isolated from the airways of children with CF as part of a natural history study. Common characteristics of bacteria airway adaptation will be defined using biochemical and phenotypic characterization as well as the most modern techniques in biological research including: DNA microarray analysis, genome sequencing, and quantitative proteomic analysis with mass spectrometry. This proposal will establish prevalence and clinical correlations for the characteristics associated with adaptation to the CF airway by performing cross-sectional and longitudinal studies on CF children with disease of varying severity. The data obtained will test the hypothesis: that characteristics of *P. aeruginosa* clinical isolates from children with CF can function as markers to predict clinical outcome and therapeutic response. Furthermore, the knowledge obtained from this proposal may direct the development of new therapeutic interventions for CF patients.

**Grant:** 1R01DK066288-01  
**Program Director:** KARP, ROBERT W  
**Principal Investigator:** FRANCINO, M PILAR PHD  
**Title:** Comparative Community Genomics of the Gut Microbiota  
**Institution:** UNIVERSITY OF CALIF-LAWRENC BERKELEY BERKELEY, CA  
LAB  
**Project Period:** 2004/07/01-2008/02/29

**DESCRIPTION** (provided by applicant): The global aim of this project is to increase our understanding of the diverse microbial community that inhabits the human gastrointestinal (GI) tract. This microbiota plays essential roles in human health, including a significant contribution to the digestive process, promotion of gut maturation and integrity and modulation of the immune system. Moreover, the microbiota interacts with pathogenic agents in several complex ways. On one hand, resident bacteria exert a protective barrier effect against enteropathogens; but, on the other, they could contribute to enrich the arsenal of incoming pathogens through horizontal transmission of genes involved in host-microbe interaction or antibiotic resistance. In addition, many normally benign GI commensals have the potential to become opportunistic pathogens in compromised hosts. Elucidating the composition and coding capabilities of the GI microbiota is therefore crucial for a comprehensive analysis of infectious disease. To advance towards this goal, we will produce large-insert bacterial artificial chromosome (BAC) libraries from genomic DNA isolated directly from fecal samples. The availability of BAC libraries will allow for a deep characterization of the GI microbiota by providing extensive genomic sequences that will serve to elucidate the coding capabilities as well as the phylogenetic positions of the members of this community. Given that the composition of the GI microbiota varies greatly with age and diet, we have chosen to generate BAC libraries from two very distinct stages of microbiota development: adults and breast-feeding infants, as represented by mother and child. Because of the widespread use of mice as an experimental system to study both infectious diseases in general and the GI microbiota in particular, we will also generate BAC libraries for mouse mother and suckling pup. To maximize our insight into the evolution and ecology of infectious disease, we will focus our sequencing efforts towards genomic regions relevant to pathogenicity and other ecological interactions, both among the microbial community members and between microbes and host.

**Grant:** 1R01DK067928-01  
**Program Director:** HAMILTON, FRANK A.  
**Principal Investigator:** ENGELHARDT, JOHN F PHD  
**Title:** Redox-mediated NF-kappaB activation by LPS and IL-1  
**Institution:** UNIVERSITY OF IOWA IOWA CITY, IA  
**Project Period:** 2004/06/01-2009/05/31

DESCRIPTION (provided by applicant): Systemic multi-organ injury is an important component associated with sepsis. Multi-organ dysfunction during sepsis can be caused by a myriad of factors, including endotoxin and systemic production of proinflammatory cytokines, such as TNFalpha and IL-1beta. The liver is a major source of cytokine expression in response to endotoxin and hence plays a critical role in mediating systemic organ injury during sepsis. Hepatic responses to LPS that control proinflammatory cytokine production include a number of receptor-mediated signal transduction pathways, of which NF-kappaB is one of the most important. In this context, NF-kappaB activation in the liver directly controls the induction of TNFalpha in response to LPS and mediates signals that are important to hepatic cell survival. We have determined that IL-1beta and LPS share interesting similarities and differences in their mechanisms of NF-kappaB activation. Both involve redox-sensitive pathways that appear to be controlled by NADPH oxidase activation and converge at the level of the IKB kinase (IKK) complex. LPS and IL-1beta activation of NF-kappaB also utilize two related receptors (TLR4 and IL-1R) that share similar effector complexes. Despite the similar dependence of both these pathways on the intracellular production of reactive oxygen species (ROS), LPS and IL-1beta activate NF-kappaB through distinct subunits of the IKK complex. The manner in which ROS uniquely control NF-kappaB activation by LPS and IL-1beta remains unclear. This project proposes to dissect the redox-mediated events that control these activation pathways using both in vitro and in vivo mouse models. In vitro studies will evaluate LPS and IL-1beta responses in hepatocytes and Kupffer cell line models in order to identify the redox-regulated components of the TLR4 and IL-1R receptor complexes that mediate activation of specific IKK kinases. An important aspect of these mechanisms involves the formation of redox-active endosomes (termed redoxosomes) that appear to cluster ligand-activated receptors into NADPH oxidase active endosomes. We hypothesize that redoxosomes help partition ROS to redox-dependent TLR4 and IL-1R effector domains. Through this process, endosomal compartmentalization of ROS allows only ligand-activated receptor/effector complexes to be influenced by NADPH oxidase. In vivo studies will utilize mouse models of endotoxemia to study redoxosomal functions in vivo and their contribution to hepatic NF-kappaB activation pathways. Findings from these studies may lead to alternative therapeutic approaches targeting the liver by which to abrogate the detrimental effects of systemic cytokine production during sepsis.

**Grant:** 1R01DK068324-01  
**Program Director:** HAMILTON, FRANK A.  
**Principal Investigator:** DUDEJA, PRADEEP K PHD BIOCHEMISTRY  
**Title:** Modulation of NHE Activity by EPEC Infection  
**Institution:** UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL  
**Project Period:** 2004/07/15-2009/06/30

DESCRIPTION (provided by applicant): Enteropathogenic E. coli (EPEC), an important food-borne pathogen, causes infantile diarrhea resulting in significant morbidity and mortality. However, the mechanisms of EPEC-induced diarrhea remain unclear. Diarrhea results from either increased secretion, decreased absorption, or both. Earlier studies have failed to conclusively show an increase in host secretory response by EPEC infection. Our preliminary data utilizing Caco-2 cells, showed that EPEC infection significantly inhibited the activities of both the predominant Na<sup>+</sup> absorbing isoform NHE3 and the Cl-OH<sup>-</sup> exchanger. An analysis of the EPEC effects on NHEs showed that NHE1 and NHE2 activities were rapidly increased while the activity of NHE3 was significantly decreased. We hypothesize that one of the potential mechanisms of EPEC-induced diarrhea involves a decrease in NaCl absorption in the intestine. Our proposed studies will explore the effects of EPEC infection on intestinal NHE activities both in in vitro and an in vivo model and elucidate the mechanisms(s) underlying the differential regulation of NHEs by EPEC. Our Specific Aims are designed to: 1. Determine the effects of infection by EPEC and select mutants on NHE (1,2 and 3) activities and Na<sup>+</sup> flux in model human small intestinal (Caco-2) and colonic (T84, NCM460) epithelia, 2. Define mechanisms of EPEC-induced modulation of NHE isoform activities by elucidating signaling pathways involved, the role of regulatory factors NHERF1 and NHERF2), the role of the cytoskeletal protein ezrin, and NHE membrane trafficking; 3. examine the effects of EPEC on Na<sup>+</sup> transport in ileum and colon in murine model of EPEC infection by determining the expression and activities of NHEs, transepithelial Na<sup>+</sup> fluxes, gut luminal fluid accumulation, and the role of EPEC virulence genes. Results from these studies will enhance our understanding of the mechanisms of regulation of human intestinal NHEs and their modulation by pathogenic organisms. Our findings may aid in the future development of improved therapeutic modalities for infectious diarrhea.

**Grant:** 1R01DK068585-01  
**Program Director:** MULLINS, CHRISTOPHER V.  
**Principal Investigator:** MULVEY, MATTHEW A BS  
**Title:** Bacterial Invasion and Trafficking within the Bladder  
**Institution:** UNIVERSITY OF UTAH SALT LAKE CITY, UT  
**Project Period:** 2004/06/01-2009/05/31

**DESCRIPTION** (provided by applicant): Strains of uropathogenic *Escherichia coli* (UPEC) are the primary causative agents of urinary tract infections (UTIs). UPEC isolates are able to transiently invade, survive and multiply within host cells and tissues within the urinary tract. This is potentially critical to the ability of UPEC to effectively colonize the urinary tract and may promote the establishment of long-term, low-level infections within the bladder. Such sub-clinical infections may serve as a source for the recurrent acute UTIs that plague many individuals throughout their lives. The primary objectives of this proposal are to define the mechanisms by which UPEC is able to move into the bladder epithelium, persist, multiply and eventually reemerge. Using cell culture and mouse UTI model systems, the host receptors and signaling pathway utilized by UPEC for entry into bladder epithelial cells will be defined. The capacity of the host actin cytoskeleton to modulate UPEC replication and reemergence will be determined. In addition, the ability of UPEC to hijack the host cell vesicular trafficking and sorting machinery will be assessed. This research will provide a greater understanding of UTIs, which presently rank among the most common of bacterial infections, and will contribute to the development of more efficacious antibacterial therapeutics.

**Grant:** 2R15DK058128-03  
**Program Director:** MCKEON, CATHERINE  
**Principal Investigator:** YU, HONGWEI PHD  
**Title:** Genetic basis of *P. aeruginosa* lung infections in mice  
**Institution:** MARSHALL UNIVERSITY HUNTINGTON, WV  
**Project Period:** 2000/07/01-2006/03/31

DESCRIPTION (provided by applicant): Cystic fibrosis (CF) is an autosomal recessive lethal disease that mainly affects the Caucasian populations. Chronic lung infections with *Pseudomonas aeruginosa* is the leading cause of the morbidity and mortality in CF. Although CFTR mutations predispose the host with increased susceptibility to this and other bacterial pathogens, it is still unclear what innate susceptibility mechanisms are required for the establishment of initial phase of bacterial lung colonizations. To better understand the etiology of bacterial lung infections in conjunction with genetic defects in CF, we identified a *P. aeruginosa*-susceptible mouse strain and will use this inbred mice to map and identify a mouse susceptibility gene. Using a bacterial aerosol infection mouse model, we screened seven inbred mice for the altered susceptibility to *P. aeruginosa* and *S. aureus* lung colonizations. DBA/2 mice were extremely susceptible to lung colonizations by both CF-relevant pathogens while C57BL/6, C3H/HeN, and NJ mice displayed a resistant phenotype. This susceptibility trait is autosomal recessive and seems to be controlled by a single allele. While DBA/2 neutrophils were bactericidal, the delay of neutrophil infiltrations to the lungs in response to bacterial aerosol challenges appeared to be associated with increased susceptibility. In this proposal, we will further examine the genetic basis of bacterial lung infections in mice by pursuing the following two specific aims: 1) to map a mouse susceptibility locus to *P. aeruginosa* and *S. aureus* lung infections; and 2) to identify the components of innate lung defenses associated with the *P. aeruginosa* and *S. aureus* clearance. We also plan to produce a congenic CFTR mutant mouse in DBA/2 background to test whether homozygous CFTR mice carrying *P. aeruginosa*-susceptible allele will naturally acquire lung infections as seen in humans with CF. The discovery of the mouse susceptibility gene may lead to the identification of the human homologue (lung-specific modifier gene) presumed to mediate susceptibility in the initial phase of infection.



**Grant:** 1R21DK065768-01  
**Program Director:** HAMILTON, FRANK A.  
**Principal Investigator:** COLGAN, SEAN P PHD  
**Title:** Epithelial BPI and intestinal inflammation  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 2004/06/01-2006/05/31

**DESCRIPTION** (provided by applicant): The human intestine is lined by a single layer of protective epithelial cells, which possess properties such as barrier and ion transport functions. In addition, the epithelium functions as a component of the innate immune system, including expression of functional antimicrobial peptides. Recent studies from our laboratory indicate that intestinal epithelial cells express the anti-infective molecule bactericidal permeability-increasing protein (BPI), a peptide with potent antimicrobial and endotoxin-neutralizing activity. Preliminary studies for this application indicate that: a) intestinal epithelial BPI is functionally important in regulating epithelial responses to endotoxin; b) that bacterial-derived products can regulate epithelial expression of BPI; and c) that the murine homolog of BPI is expressed on intestinal epithelia. In the proposed studies we will systematically define activation pathways of epithelial BPI production and define regulatory pathways both in vitro and in vivo. Parallel experiments will be done utilizing epithelial cell lines and native human intestinal tissue to define these principles. First, we will elucidate the basis of epithelial BPI production. Using molecular, morphologic and functional readouts, we will elucidate molecular pathways of epithelial BPI expression. Second, we will define the role of epithelial BPI in murine colitis. Third, we will profile the expression patterns of epithelial BPI protein and mRNA in patients with inflammatory bowel disease (IBD). Successful completion of the Aims outlined in this application will yield insight into the role of endogenous antimicrobial peptides in the pathogenesis of IBD and will provide predictive value for future development of experimental therapeutics for such disorders.

**Grant:** 1R21DK067065-01  
**Program Director:** KARP, ROBERT W  
**Principal Investigator:** GUILLEMIN, KAREN J BA  
**Title:** The role of indigeous Microbiota in development of the zebrafish GI tract  
**Institution:** UNIVERSITY OF OREGON EUGENE, OR  
**Project Period:** 2004/09/01-2006/06/30

DESCRIPTION (provided by applicant): All animals coexist with microbes, often in intimate and specific associations. The indigenous microbiota of mammals plays important roles in the development and function of the digestive and immune systems. Many of the studies on the role of the normal microbiota has been conducted in rodent models, using gnotobiology to investigate morphological and physiological differences between conventionally and sterilely reared (germ-free) animals. While the gnotobiotic mouse has been a powerful system in which to examine this question, it has several limitations. First, the cost and expertise required to maintain this model have limited its use to a handful of research centers worldwide. Second, the mouse model is not amenable to large-scale screens to explore the host factors involved in bacterial-host interactions. In this R21 grant proposal for innovative and exploratory research in digestive diseases and nutrition, we propose to develop the zebrafish, *Danio rerio*, as a system to study the role of the microbiota in development of the gastrointestinal tract. The experiments proposed will establish the groundwork for a system in which we can explore the molecular genetics of bacterial-host interactions in gut development, taking advantage of all of the tools offered by this model vertebrate host including forward genetics and genomics. Specifically, we propose to ask: 1). What bacteria are normally associated with laboratory-reared zebrafish during development? 2). Can zebrafish be reared germ-free? 3). Are there phenotypic differences between conventionally reared and germ-free zebrafish? 4). Is colonization of germ-free zebrafish with bacteria sufficient to reverse any of the germ-free phenotypes? By establishing this vertebrate model system, we will be able to experimentally address questions important for understanding and treating human diseases associated with imbalances in and inappropriate responses to the indigenous microbiota, such as opportunistic infections, inflammatory bowel disease, and atopic allergies.

**Grant:** 1R21DK067212-01  
**Program Director:** HAMILTON, FRANK A.  
**Principal Investigator:** TOBACK, FREDERICK G  
**Title:** A Novel Cytoprotective Peptide for GI Epithelial Cell  
**Institution:** UNIVERSITY OF CHICAGO CHICAGO, IL  
**Project Period:** 2004/04/15-2006/03/31

DESCRIPTION (provided by applicant): Disruption of intestinal barrier function may be a common pathogenetic mechanism that mediates morbidity and mortality in subjects with inflammatory bowel disease (IBD) and gut-derived sepsis (GDS). If so, a therapeutic strategy that protects mucosal barrier function and structure by increasing accumulation of tight and adhesion junction proteins that connect mucosal epithelial cells could be used to treat these conditions. We recently characterized a novel 18-kDa protein called AMP-18, that is synthesized in epithelial cells of the gastric antrum mucosa of humans and 6 other mammals, and has mitogenic and motogenic properties. A 21-mer peptide derived from the central domain of the protein was found to be cytoprotective in human colonic epithelial cell (Caco-2/bbe) cultures subjected to injury by an oxidant, indomethacin, or dextran sulfate sodium (DSS) used to induce colitis in mice. Studies in cell culture indicate that this AMP peptide activates p38 MAP kinase, increases accumulation of specific tight junction proteins (occludin, ZO-1, claudin-5), an adherens junction protein (E-cadherin), and heat shock proteins (hsp25, hsp72), and protects the actin microfilament network in cells exposed to cytochalasin D. When given to mice, AMP peptide increases the content of hyperphosphorylated occludin in the colonic mucosa. To determine if these biological effects of AMP peptide confer colonic cytoprotection in vivo, its effectiveness was tested in two important animal models of barrier compromise, IBD and GDS. Pretreatment of mice with AMP peptide delayed the onset of bloody diarrhea and reduced weight loss in animals given DSS to induce colitis. The peptide also prevented the death of mice given intracecal *Pseudomonas aeruginosa* following the surgical stress of partial hepatectomy and post-operative food deprivation in a model GDS. Our objective is to obtain additional support in cell culture and in vivo models of IBD and GDS to demonstrate that AMP peptide, by increasing accumulation of tight junction proteins such as occludin, protects colonic mucosal barrier structure and function. Studies that confirm and extend the cytoprotective, mitogenic, and motogenic effects of AMP peptide would lend further support to developing this molecule as a therapeutic agent to treat not only IBD and GDS, but other GI diseases mediated by disruption of intestinal tight junctions and the subsequent increase in mucosal permeability to bacteria and their products.

**Grant:** 1R21DK067965-01  
**Program Director:** SERRANO, JOSE  
**Principal Investigator:** DAWSON, PAUL A PHD  
**Title:** Host-Microbial Control of Deoxycholate Production  
**Institution:** WAKE FOREST UNIVERSITY HEALTH SCIENCES WINSTON-SALEM, NC  
**Project Period:** 2004/09/01-2006/06/30

**DESCRIPTION** (provided by applicant): Elevated deoxycholate production is a positive risk factor for the development of colon cancer as well as cholesterol gallstones. However, the factors that regulate deoxycholate production are poorly understood. Only a few bacterial strains (specific members of the Eubacterium and Clostridium genera) carry the bile acid inducible (bai) operon that encodes the enzymes responsible for bile acid 7 $\alpha$ -dehydroxylation, and these organisms constitute only a tiny fraction of the intestinal flora. While deoxycholate production correlates directly with intestinal flora content of bai operon-containing bacteria, little is known about what factors control the intestinal content of these organisms. We have recently developed a mouse model of primary bile acid malabsorption, the ileal bile acid transporter (Slc10a2) knockout mouse. Slc10a2 null mice in a 129S6/SvEv background develop severe bile acid malabsorption. In contrast, crossing the Slc10a2 null allele into a C57BL/6J background produced only bile acid malabsorption accompanied by increased deoxycholate production. This is the first example of the host genetic background apparently regulating the colonic content of deoxycholate-producing bacteria. The goal of this R21 application is to develop a mouse model system to study the genetic and environmental factors that regulate deoxycholate production. Three specific aims are proposed. Aim 1: To test the hypothesis that differences in the gut flora deoxycholate production are responsible for bile acid absorption differences between mouse strains lacking the ileal bile acid transporter (Slc10a2). The mouse strain gut flora differences will be assessed using antibiotics and bile acid analogs to measure gut flora differences in deoxycholate production. Aim 2: To develop enzyme and PCR-based assays to directly quantify the gut flora bacterium harboring the bai operon. Many of the genes responsible for the complex pathway that converts cholate to deoxycholate are located on a single bile acid inducible (bai) operon. In human colonic and fecal samples, cholic acid 7 $\alpha$ -dehydroxylation activity has been directly measured and the bai genes detected by PCR. The goal of this aim is to apply these assays to the mouse to quantify host strain differences in bile acid 7 $\alpha$ -dehydroxylation by the enteric flora. Aim 3: To cross the Slc10a2 null allele into additional mouse strains as a first step toward cloning the genes responsible for controlling deoxycholate production (colonization by the bai operon bacteria).

**Grant:** 1R21DK069526-01  
**Program Director:** MULLINS, CHRISTOPHER V.  
**Principal Investigator:** MULVEY, MATTHEW A BS  
**Title:** Control of bladder infections by NF-kappaB  
**Institution:** UNIVERSITY OF UTAH SALT LAKE CITY, UT  
**Project Period:** 2004/09/30-2006/08/31

DESCRIPTION (provided by applicant): Urinary tract infections, including cystitis and pyelonephritis, are among the most common of infectious diseases acquired by humans. These infections are responsible for substantial morbidity, mortality and high medical costs worldwide. An especially troublesome aspect of these infections is their strong tendency to recur. The vast majority of urinary tract infections are caused by strains of uropathogenic Escherichia coli (UPEC) that can bind and invade bladder epithelial cells. Within bladder cells, UPEC will either multiply or enter a non-replicating, persistent quiescent state that may serve as a reservoir for future recurrent acute infections. Intracellular UPEC is protected against standard antibiotic treatments that can effectively sterilize the urine. In addition, resistance to multiple antibiotics among UPEC isolates is becoming increasingly widespread, further limiting the efficacy of current treatment protocols for urinary tract infections. Using a cell culture model system, we recently found that inhibitors of the host transcription factor NF-kappaB are able to effectively interfere with the ability of UPEC to invade bladder epithelial cells, multiply intracellularly and reemerge from the host cells. NF-kappaB regulates the transcription of a number of host genes, including many associated with pro-inflammatory responses that work to limit infections. Our preliminary data, however, suggest the counterintuitive possibility that NF-kappaB activity is required for UPEC to effectively colonize and persist within host bladder epithelial cells. The primary Aim of this R21 application is to define the role of NF-kappaB as a regulator and potential facilitator of UPEC pathogenesis. In addition, we will test the therapeutic potential of a NF-kappaB inhibitor in the treatment and prevention of both acute and recurrent bladder infections using a well-established mouse cystitis model.

**Grant:** 4R37DK050814-29  
**Program Director:** NYBERG, LEROY M.  
**Principal Investigator:** ABRAHAM, SOMAN N PHD  
**Title:** LIGAND RECEPTOR INTERACTIONS IN UTIS  
**Institution:** DUKE UNIVERSITY DURHAM, NC  
**Project Period:** 1976/08/01-2009/06/30

DESCRIPTION (Adapted from the Applicant's Abstract): Urinary tract infections (UTIs) are a major cause of morbidity in women and the aged. Over 80% of UTIs are caused by *E. coli* and most of these strains express the type 1 fimbriae. Since these fimbriae are also expressed on nonpathogenic *E. coli* their role in virulence is unclear. However, the investigators have detected considerable heterogeneity in binding among different type 1 fimbriae which could determine to which strains are virulent and the nature of the inflammatory response in the host. MC are prominent inflammatory cells in the bladder and are found in close proximity to the bladder epithelial cells (BEC). they have also found that certain type 1 fimbriated strains of *E. coli* can gain access into mast cells (MC) and then subsequently escape without loss of bacterial viability. The trafficking process is triggered by the specific binding of type 1 fimbriae to CD48 on the MC membrane. Interestingly, BEC were found to modulate the trafficking of *E. coli* in MC as a reservoir may in release of stem cell factor (SCF). This capacity of type 1 fimbriated *E. coli* to utilize MC as a reservoir may in part explain recurrent UTIs. To extend their observations they plan to (i), determine if and how type 1 fimbriae from uropathogenic *E. coli* isolates display distinct binding traits compared to commensal isolates (ii), determine whether different *E. coli* type 1 fimbriae bind separate receptors on the host cells and if there is diversity in the host cell's responses (iii), elucidate the molecular mechanisms of CD48 mediated trafficking of type 1 fimbriated *E. coli* in MC and (iv), elucidate how SCF modulates MC carriage of bacteria. The information derived from these studies could promote the development of novel strategies for the prevention and management of *E. coli* -induced UTIs including recurrences. In the age of increasing antibiotic resistance in bacteria and where the population of the aged and immunocompromised individuals continues to grow, these studies could prove to be extremely valuable.

**Grant:** 2R01EB001035-19A1  
**Program Director:** MCLAUGHLIN, ALAN  
**Principal Investigator:** HERZFELD, JUDITH PHD CHEMISTRY:PHYSICAL  
**Title:** NMR Studies of Biological Membranes  
**Institution:** BRANDEIS UNIVERSITY WALTHAM, MA  
**Project Period:** 1985/09/30-2008/07/31

DESCRIPTION (provided by applicant): Ion pumps, which maintain the internal milieu of cells and control the potentials of cell membranes, have demanding requirements with respect to the timing and vectoriality of their action. In the proposed work we will compare the manner in which these requirements are met by three different light-driven ion pumps, a green-driven proton/hydroxyl pump (wild type bacteriorhodopsin), a green-driven halide pump (acid purple bacteriorhodopsin), and a blue-driven proton/hydroxyl pump (D85N bacteriorhodopsin at high pH). Our hypotheses are that (1) chromophore distortion and relaxation are central to the action of bacteriorhodopsin and (2) there is no need for such complications in the other two pumps. The pump mechanisms are to be studied by SSNMR using in situ illumination to accumulate critical photocycle intermediates and low temperature to stabilize them during data acquisition. Samples will be uniformly and selectively labeled with  $^{13}\text{C}$  and  $^{15}\text{N}$  as needed. Dynamic nuclear polarization (DNP) will be used to enhance signals, thereby (1) greatly expediting measurements of internuclear distances in the active site and (2) extending the range of feasible experiments to include sensitive measurements of torsion angles along the chromophore. The evolution of these variables over the course of each photocycle will provide insight into the mechanism by which vectorial action is enforced in the system. In addition, REDOR and high-field MQMAS will be used to study the interactions of deuterons and chloride with elements lining the transport channels of the proton/hydroxyl and chloride pumps, respectively. The evolution of these interactions during the photocycle will provide a picture of substrate movement through the pump. Interpretations of chemical shifts in terms of local structure will be assisted by ab initio density functional calculations and NMR-derived distances and angles will be compared with crystallographic results. Taken together, the experiments will significantly enhance our understanding of ion pump function and dysfunction.

**Grant:** 1R01EB003824-01  
**Program Director:** KORTE, BRENDA  
**Principal Investigator:** NOLAN, JOHN P PHD  
**Title:** Raman Flow Cytometry for Diagnostics and Drug Discovery  
**Institution:** LA JOLLA BIOENGINEERING INSTITUTE LA JOLLA, CA  
**Project Period:** 2004/09/01-2009/08/31

DESCRIPTION (provided by applicant): The ability to make quantitative, high throughput molecular measurements of biological systems is a critical need for many areas of biomedical research. This Bioengineering Research Partnership (BRP) aims to develop a powerful new analytical platform for high throughput screening and selection based on Raman Flow Cytometry. This Partnership will develop new analytical instrumentation, optically encoded polymer resins for chemical synthesis and screening, and nanostructured materials with unique optically properties for sensitive reporting and encoding. The new technology will perform Raman spectroscopy on single particles in flow to enable new applications in sensitive multiplexed detection, drug discovery, and diagnostics. The Raman Flow Cytometry instrumentation, and applications will be developed by a Partnership involving engineers, biologists, and chemists from academia, government and industry. In the first year of the Partnership, we will modify a commercial particle sorter to detect individual Raman vibrational bands from single particles and sort these particles based on their optical signature. In Years 2-5, we will develop the ability to collect and analyze complete Raman spectra from single particles. In parallel, the Partnership will develop new encoding and reporting strategies for multiplexed molecular analysis and separation. This Raman Flow Cytometry technology will be applied to the development of therapeutics and diagnostics for bacterial pathogens and their toxins. Raman Flow Cytometry will be an important and general new analytical and separation capability that will impact many areas of basic and applied biomedical research in addition to the applications proposed here.



**Grant:** 1R21EB004165-01  
**Program Director:** MOY, PETER  
**Principal Investigator:** FENNELLY, GLENN J MD  
**Title:** Nonpathogenic Mycobacteria:Anti-Bladder Tumor Therapy  
**Institution:** YESHIVA UNIVERSITY BRONX, NY  
**Project Period:** 2004/07/01-2006/06/30

**DESCRIPTION** (provided by applicant): This R21 proposal is submitted in response to PA-03-058 Exploratory/Developmental Bioengineering Research Grants. Our overall objective is to engineer a novel anti-tumor therapy with eukaryotic expression plasmids that encode therapeutic genes delivered in nonpathogenic mycobacteria, as a safer alternative to viral gene vectors. In particular, we will study the efficacy of wild type *Mycobacterium smegmatis*, and *Mycobacterium bovis* BCG, or alternative modified mycobacteria, for the delivery of genes that express functional cytokines or co-stimulatory molecules to bladder tumor cells. Intravesicular BCG therapy, the only US FDA-approved antitumor microbial agent in the US, has contributed to a > 20% decline in death rates from bladder cancer since 1980. Intravesicular BCG augments local production of immune mediators of tumor clearance (IFN-gamma, ICAM-1 and TNF-alpha) and has direct anti-tumor activity. Nevertheless, BCG has no effect in > 20% of cases. *M. smegmatis*, a species that is less virulent than BCG, inhibits tumor cell growth in vitro more potently than BCG. Previous studies by us, and others, demonstrate that both *S. flexneri* and *S. typhimurium* can deliver plasmids to eukaryotic cells for genetic immunization. In a set of pilot experiments, we tested the ability of *M. smegmatis* to deliver eukaryotic expression plasmids to mammalian cells and clearly demonstrated, for the first time, that *M. smegmatis* can deliver plasmids expressing the green fluorescent protein from a eukaryotic promoter to macrophages. Using this discovery, we plan to develop *M. smegmatis* and BCG as safe and efficient vectors for the delivery of ICAM-1 and TNF-alpha eukaryotic expression plasmids as gene therapy against bladder carcinoma in humans. Specifically, we propose to: 1. Optimize the ability of wild type *M. smegmatis* and BCG to deliver Mycobacterial Mammalian Shuttle plasmids (MMSP) to macrophages and murine or human bladder tumor cells in vitro and in mice. 2. Determine whether infection of murine bladder tumor cells with recombinant Mycobacteria harboring MMSP that encode murine ICAM-1 or TNF-alpha augment tumor cell expression of functional ICAM-1 or TNF-alpha in vitro and, 3. Evaluate the ability of recombinant Mycobacteria harboring MMSP that encode ICAM-1 or TNF-alpha genes to enhance tumor regression in a murine MB49 syngeneic orthotopic bladder cancer model using C57BL/6 (immunocompetent).

**Grant:** 2R01ES009882-04A1  
**Program Director:** TINKLE, SALLY S.  
**Principal Investigator:** FLOROS, JOANNA  
**Title:** Ozone Effects on Function of Surfactant Protein Variants  
**Institution:** PENNSYLVANIA STATE UNIV HERSHEY MED HERSHEY, PA  
CTR  
**Project Period:** 1999/05/01-2009/07/31

**DESCRIPTION** (provided by applicant): Published work points to an important role of surfactant protein A (SP-A) in innate host defense. SP-A modulates a number of host defense processes and exposure to ozone results in functional and structural alterations of SP-A. Two human genes, SP-A1 and SP-A2, and several genetic variants for each SP-A gene have been characterized. In vitro studies show functional, structural, or biochemical differences between the two genes and the gene-specific variants. SP-A levels are altered in a variety of lung diseases, and SP-A genetic variants are associated with risk for several pulmonary diseases. Our central hypothesis is that differences among SP-A variants account for differences in risk to lung disease in response to environmental insults. Our specific hypothesis is that the two human SP-A gene products are not functionally equivalent with regards to their host defense function, and that ozone exposure has a differential impact on this function. To investigate the specific hypothesis, we propose to study the effect of ozone, on pathogen-infected C57BL/6 and SP-A<sup>-/-</sup> mice (Aim 1) by assessing: a) survival with bacterial infection; b) ability to clear pathogens from their lungs and limit dissemination of infection; c) cytokine production, the in vivo phagocytic index of macrophages, and the in vivo oxidation status of SP-A; as well as, on the ex vivo host defense function of macrophages from wild type and SP-A<sup>-/-</sup> mice (Aim 2). To generate transgenic mouse lines on the SP-A<sup>-/-</sup> background that express equivalent levels of human SP-A1 or SP-A2 gene products (Aim 3). To study the impact of ozone on the SP-A1 and SP-A2 transgenic mouse lines by carrying out studies similar to those described for Aim 1 and by studying biochemical characteristics of the transgene products (Aim 4), and the ex vivo host defense function of macrophages from the SP-A1 and SP-A2 mouse lines (Aim 5). Through the proposed work we will generate an animal model to study human SP-A variants, and we will determine, in vivo, and in response to ozone exposure, the role of SP-A in host defense and assess functional host defense differences between the two human SP-A gene products. Knowledge gained may help explain and perhaps link the in vitro data with the human genetic association data, and provide insight in the understanding of the value of the SP-A gene duplication.

**Grant:** 1R01ES012706-01A1  
**Program Director:** TINKLE, SALLY S.  
**Principal Investigator:** PEDEN, DAVID B MD  
**Title:** Ozone and LPS-Induced Airway Inflammation In Humans  
**Institution:** UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC  
HILL  
**Project Period:** 2004/09/01-2009/07/31

DESCRIPTION (provided by applicant): Ozone (O<sub>3</sub>) and particulate matter (PM) are the most commonly encountered air pollutants in the United States. Exposure to even low levels of these pollutants has been associated with increased hospitalizations for respiratory disorders, exacerbations of asthma and other respiratory tract diseases. Coarse and fine mode PM is comprised of a number of classes of components, including biologicals, such as endotoxin (or lipopolysaccharide or LPS). Though regulated and considered on an individual basis, people often encounter increased exposure to O<sub>3</sub> and LPS simultaneously with low level exposures causing exacerbation of lung disease. A number of observations suggest that LPS and O<sub>3</sub> share a number of general mechanistic features, which mediate induction of neutrophilic inflammation. We have observed that expression of CD11b on circulating monocytes (and to lesser extent neutrophils) correlates very well with neutrophil influx to the airway following challenge with either agent. Others have observed that the toll-like receptor 4 (TLR4, which along with CD14 is a primary receptor for LPS) has been implicated in the airway response of rodents to ozone. The inflammatory nature of the response to both O<sub>3</sub> and LPS, taken together with the observations outlined above, suggests that common determinants of susceptibility account for increased responsiveness to both stimuli. Animal studies suggest that ozone modifies the response to LPS. In humans, we have found that challenge with low levels of LPS can enhance macrophage responsiveness, and suspect that low level O<sub>3</sub> may act similarly. Taken together, these observations lead to the following hypotheses: 1. That inflammatory response to ozone will correlate with response to LPS, suggesting a common airway inflammation response phenotype; 2. That low-level O<sub>3</sub> exposure enhances macrophage responsiveness to inflammatory stimuli, and; 3. That low level ozone will enhance response to LPS. Testing these hypotheses will be used to better define the ways that O<sub>3</sub> and LPS interact to exacerbate airway disease.

**Grant:** 1R01ES013265-01  
**Program Director:** LAWLER, CINDY P  
**Principal Investigator:** LIU, BIN PHD  
**Title:** Combined dopaminergic neurotoxicity of manganese and LPS  
**Institution:** UNIVERSITY OF FLORIDA GAINESVILLE, FL  
**Project Period:** 2004/08/01-2007/04/30

DESCRIPTION (provided by applicant): Parkinson's disease (PD) is a movement disorder characterized by a progressive and selective loss of dopamine (DA) neurons in the substantia nigra. At the present, PD affects nearly one million people in the US alone and the incidence is expected to increase with an aging population. The vast majority of PD is idiopathic, occurs late in life, and does not appear to be directly related to gene mutations identified in several familial clusters of early-onset PD. Accumulating evidence indicates that environmental agents including heavy metals and infectious agents represent risk factors for idiopathic PD. The DA neurotoxicity of the heavy metal manganese has been documented and activation of brain immune cells (microglia and astroglia) by bacterial endotoxin lipopolysaccharide (LPS) is known to release neurotoxic factors to induce neurodegeneration. However, the etiology of PD may be multi-factorial: the development of PD may be a final outcome of chronic exposure to low concentrations of multiple environmental agents. Therefore, this proposal plans to study the combined DA neurotoxicity of low concentrations of the heavy metal manganese and LPS in a chronic primary neuron-glia culture-based model of PD. The mechanism of the combined neurotoxicity will be analyzed at both the cellular and molecular levels. At the cellular level, the relative contribution to the combined neurotoxicity of the primary brain immune cells, microglia, and astroglia will be determined. At the molecular levels, the contribution to neurodegeneration of neurotoxic factors (free radicals and cytokines) by activated glia and the mechanism of neurodegenerative process will be examined. The results of these studies will enable us to gain understanding of the impact of heavy metals and microbial toxins on the DA system. These studies will also be an important component of our long-term goal of understanding the interaction between environmental factors and the nervous system in relation to the etiology of PD and finding effective strategies for the prevention and/or treatment of the disease.

**Grant:** 2R01EY002986-26  
**Program Director:** SHEN, GRACE L  
**Principal Investigator:** HAZLETT, LINDA D PHD BIOLOGY NEC:BIOLOGY  
NEC-UNSPEC  
**Title:** Alteration with Age of Resistance to Eye Infections  
**Institution:** WAYNE STATE UNIVERSITY DETROIT, MI  
**Project Period:** 1979/04/01-2009/03/31

DESCRIPTION (provided by applicant): *Pseudomonas aeruginosa* (*P. aeruginosa*) is a common organism associated with bacterial keratitis, especially in more tropical climates and in extended wear contact lens users. The incidence of microbial keratitis is 25,000-30,000 cases annually with cost of treatment estimated at \$15-30 million, making the disease of considerable medical and economic impact. In the studies proposed, we will test the overall hypothesis that after corneal infection with *P. aeruginosa*, the neuropeptides VIP/PACAP inhibit/downregulate sustained pro-inflammatory cytokine and nitric oxide (NO) production and upregulate anti-inflammatory cytokine production, leading to the resistance response of BALB/c mice. A corollary to this hypothesis is that inflammatory neuropeptides such as SP/CGRP will have a converse effect, leading to the susceptible response of B6 mice. The aims of this proposal are: 1) to test the hypothesis that the distribution of neuropeptides VIP/PACAP, SP and CGRP in the cornea will differ both spatially and temporally in BALB/c (resistant) vs. B6 (susceptible) mice after infection with *P. aeruginosa*; 2) to test the hypothesis that the neuropeptides VIP/PACAP, SP, CGRP and secretoneurin (SN) differentially affect the migration into and arrest of Langerhans cells (LC) in the infected cornea of B6 vs. BALB/c mice; 3) to test the hypothesis that SP/CGRP promote pro-inflammatory cytokine and chemokine production and the influx and persistence of PMN in the infected cornea of susceptible vs. resistant mice; 4) to test the hypothesis that in infected resistant vs. susceptible mice, VIP/PACAP inhibit sustained macrophage (Mphi) inflammatory cytokine/chemokine and NO production and enhance anti-inflammatory cytokines such as IL-10; 5) to test the hypothesis that SP/CGRP induce sustained upregulation of adhesion molecules after infection in susceptible vs. resistant mice. In the proposed studies, a combination of in vivo and in vitro systems will be used to analyze the immunomodulatory activities of these neuropeptides. It is expected that the findings will be particularly significant with respect to management of *P. aeruginosa* keratitis and should lead to better characterization of molecules which are potential targets for more effective treatment of corneal inflammation.

**Grant:** 2R01EY010320-11  
**Program Director:** SHEN, GRACE L  
**Principal Investigator:** PEARLMAN, ERIC PHD  
**Title:** The Role of Wolbachia Endobacteria in River Blindness  
**Institution:** CASE WESTERN RESERVE UNIVERSITY CLEVELAND, OH  
**Project Period:** 1993/12/01-2008/11/30

DESCRIPTION (provided by applicant): Parasitic filarial nematodes infect over 200 million individuals worldwide, causing debilitating inflammatory diseases such as river blindness and lymphatic filariasis. Rickettsia - like Wolbachia bacteria are essential symbionts of the major pathogenic filarial nematode parasites of humans, including the *Onchocerca volvulus*, which causes river blindness. Using a murine model, we reported that Wolbachia play an essential role in the pathogenesis of *Onchocerca* keratitis, primarily by mediating neutrophil recruitment to the corneal stroma [Science 2002.295:1892]. In the current proposal, we will use microfilaria larvae (Mf) which harbor Wolbachia to examine the interaction between Wolbachia and the neutrophils that lead to stromal disease, focusing on mediators of neutrophil migration through the extracellular matrix (ECM) of the corneal stroma which include CXC chemokine receptors, beta 2 integrins, and matrix metalloproteinases. The experimental plan is a logical progression from studies conducted in the previous funding period, which focused on the role of vascular endothelial cells in recruitment of neutrophils from limbal vessels to the cornea. In addition to furthering our understanding of the role of Wolbachia in the pathogenesis of river blindness, results of proposed studies represent a novel approach to define the early molecular events underlying the interaction of neutrophils with ECM that lead to development of corneal haze.

**Grant:** 2R01EY012961-05  
**Program Director:** SHEN, GRACE L  
**Principal Investigator:** O'CALLAGHAN, RICHARD J PHD  
**Title:** Mechanisms and Therapy of Bacterial Keratitis  
**Institution:** LOUISIANA STATE UNIV HSC NEW ORLEANS NEW ORLEANS, LA  
**Project Period:** 2000/05/01-2007/07/31

DESCRIPTION (provided by applicant): *Pseudomonas aeruginosa* causes a devastating form of keratitis that is typically contact lens associated. Progress in lens development to allow more oxygen penetration, according to new preliminary clinical studies, has not prevented Gram-negative bacterial keratitis. Studies of experimental *Pseudomonas* keratitis indicate that corneal damage is mediated by the action of bacterial proteins that, once released into the tissue, continue to act free of inhibition by antibiotics or other drugs now in existence. Of the multiple proteins produced by *Pseudomonas*, the one that has been best shown to be the "aggressin" that mediates corneal damage is protease IV. This protease is only produced by *P. aeruginosa* and every strain tested to date produces this enzyme. Bacterial genetic experiments have demonstrated that the production of this enzyme provides substantial corneal virulence. Given the problem of *Pseudomonas* keratitis and the proven importance of bacterial proteins in the pathogenesis, a long-term goal is to develop a means to inhibit the aggressin proteins that damage the cornea once they are produced by intra-corneal bacteria. Toward that goal, the following specific aims are proposed: 1) develop an antibody capable of neutralizing protease IV and use this antibody as passive immunization to minimize corneal damage (this approach has been effective for *Staphylococcus keratitis*); 2) quantify the contribution of other proteases, including two uncharacterized enzymes, to corneal pathology to determine if they too should be targets for protective immunotherapy; 3) determine the host molecules, including host defense molecules, that are destroyed by bacterial proteins during infection; and 4) determine the importance of specific bacterial proteins to the host responses that typify *Pseudomonas* keratitis. These host responses to be analyzed include activation of matrix metalloproteinases, production of inflammatory cytokines, and increases in corneal calcium concentration. Critical to this research are the recent development of *Pseudomonas putida* strains that produce individual gene products of *P. aeruginosa*. These engineered strains can infect and replicate in the rabbit cornea without causing corneal damage unless the cloned genes of *P. aeruginosa* are produced.

**Grant:** 2R01EY012985-05A1  
**Program Director:** SHEN, GRACE L  
**Principal Investigator:** CALLEGAN, MICHELLE C PHD  
**Title:** Pathogenesis & Chemotherapy of Bacillus Endophthalmitis  
**Institution:** UNIVERSITY OF OKLAHOMA HLTH SCIENCES OKLAHOMA CITY, OK  
CTR  
**Project Period:** 2000/06/01-2008/06/30

**DESCRIPTION** (provided by applicant): Bacillus causes one of the most explosive and devastating forms of post-traumatic or endogenous endophthalmitis that, despite aggressive antibiotic and surgical intervention, almost always results in blindness, if not loss of the eye itself. The regularity of treatment failures highlights the need for identification and characterization of the specific virulence factors involved in pathogenesis and significant improvements in existing treatment regimens. Studies conducted during the initial funding period addressed the contributions of bacterial factors to Bacillus endophthalmitis, namely bacterial cell walls, toxins, and intraocular motility. Results suggested that all factors contributed to virulence, to varying degrees. We identified the most important bacterial intraocular virulence factors to be quorum sensing regulation of toxin production and migration of Bacillus throughout the eye during infection. The most important host contributor to virulence appears to be the rapid influx of inflammatory cells into the posterior segment at the early stages of infection. These factors represent important potential targets for the development of novel therapeutics for Bacillus endophthalmitis. This proposal extends the identification of critical bacterial factors involved in virulence to identify additional therapeutic targets. To do this, we propose to analyze the virulence of strains deficient in metalloproteases, motility, or surface components, and Bacillus spores as infective agents in the eye. The proposal then shifts toward the development of improved antibiotic/anti-inflammatory therapeutic regimens for the treatment of Bacillus endophthalmitis. We propose to directly target the bacterium and the host inflammatory response during the critical, early stages of intraocular infection when initial retinal function changes are occurring. Agents designed to interfere with Bacillus growth or host inflammation will be evaluated during the course of this project, the results of which are expected to be of immediate clinical relevance in designing more rational therapeutic regimens aimed at limiting the sight-threatening consequences of intraocular infection and inflammation. These studies are a logical consequence of the original proposal, and as such will greatly advance our long-range goal of understanding the pathogenic mechanisms of disease and developing successful treatment strategies for the preservation of vision during Bacillus endophthalmitis.



**Grant:** 2R01GM018457-32A1

**Program Director:** SHAPIRO, BERT I.

**Principal Investigator:** CRAMER, WILLIAM A PHD  
BIOPHYSICS:BIOPHYSICS-  
UNSPEC

**Title:** Receptor-mediated Colicin Import

**Institution:** PURDUE UNIVERSITY WEST LAFAYETTE WEST LAFAYETTE, IN

**Project Period:** 1974/09/01-2007/11/30

**DESCRIPTION** (provided by applicant): An understanding of protein translocation and import across receptors and membranes is fundamental in biology and medicine. Receptor-mediated import of the toxin-like colicins is a paradigm for protein import to mitochondria and the import of viral proteins. Colicins E1 and E3 have different modes of bactericidal action, but parasitize the same (vitamin B12, BtuB) integral outer membrane receptor, and are used to study the mechanism of import in the E. coli inner and outer membrane, respectively. (I) OM import: (1) A 2.75 Å structure of the complex of the expressed 135 residue (R135) 100 Å long coiled-coil receptor binding domain of colicin E3, and the BtuB receptor in detergent, is the first high resolution structure of an intact integral protein receptor and its protein ligand. This structure forms the basis for studies on mechanisms of protein translocation through the outer membrane. (2) SPR and CD show that R135 binds tightly, and its receptor-distal ends unwind upon binding to BtuB. A smaller R60 peptide rewinds upon binding. The basis for these unusual properties of the binding site will be probed by mutagenesis of both R135 and BtuB, particularly the loop regions of BtuB. An R135 Arg399Ala mutant has defined a residue involved in electrostatic binding. Disulfide cross-linking of the distal ends of R135 will test the coupling between distal unwinding and binding affinity. (3) The 2.75 Å structure and the absence of large ion channels in BtuB imply that E3 does not pass through it. Occlusion of the large OmpF and TolC ion channels by E3 and E1, respectively, indicate a role for these Omp in the colicin translocon. (4) The bending and mechanical properties of the R135 coiled-coil, which might be involved in delivery of colicin domains to OmpF, will be tested by atomic force microscopy. (5) The role of two OM receptors in colicin translocation will be tested with OmpF and TolC, (i) using planar bilayers to measure changes in their channel conductance and receptor function, and the ability of individual colicin domains and TolA, TolB proteins to occlude the channels; and (ii) in co-crystallization experiments. (II) IM import: We have proposed that two prerequisites for import of soluble membrane-active proteins are segmental mobility on the membrane surface and helix extension. (1) This hypothesis will be further tested using (a) the colicin channel protein, P178, membranes of defined surface potential, and assays by FRET measurements of distance changes, and (b) solid-state NMR. (2) The pathway of membrane insertion of the E1 channel domain, relative to the time course of other unfolding events, will be examined by time-resolved FRET using a large set of single Trp mutants.

**Grant:** 2R01GM018568-32

**Program Director:** ANDERSON, JAMES J.

**Principal Investigator:** LOSICK, RICHARD M PHD  
GENETICS:BIOCHEMICAL/MOL  
ECULAR

**Title:** Role of RNA Polymerase in Bacterial Differentiation

**Institution:** HARVARD UNIVERSITY CAMBRIDGE, MA

**Project Period:** 1976/02/01-2008/01/31

DESCRIPTION (provided by applicant): The objective of this project is to elucidate the mechanisms that govern cell-specific gene expression during the process of spore formation in the bacterium *Bacillus subtilis*. Sporulation involves the formation of an asymmetrically positioned septum. The septum divides the developing cell into a forespore and a mother cell, which follow dissimilar pathways of gene expression and differentiation. Asymmetric division involves the remodeling of sister chromosomes into an elongated DNA mass that is anchored to both poles of the developing cell and a switch in the site of formation of a cytokinetic structure called the Z ring, which is composed of the tubulin-like protein FtsZ. Experiments will be carried out to investigate the role of the developmental protein RacA in chromosome remodeling and the role of the FtsZ-associated protein SpoII E in the formation of Z rings near the cell poles. The role of RacA in a checkpoint mechanism that links chromosome anchoring to polar division will also be investigated. The transcription factor sigmaF becomes active in the forespore just after the formation of the polar septum. Experiments will be carried out to investigate the coupling of sF activation to the formation of the polar septum via the SpoII E protein. Experiments will also be carried out to investigate the hypothesis that the DNA-binding protein Spo0A, which controls entry into sporulation, becomes a mother-cell-specific transcription factor after asymmetric division and sets in motion the mother-cell-line of gene expression. The mechanism by which gene expression in the mother cell is linked to gene expression in the forespore via an intercellular signal transduction pathway involving a membrane-bound proprotein processing complex will be investigated. Other projects are directed at understanding mechanisms of protein subcellular localization, elucidating on a genome-wide basis the programs of gene expression in the forespore and the mother cell, and investigating newly discovered aspects of sporulation involving multicellular interactions. These goals address basic questions of differentiation and gene control that are common to developing systems of many kinds, including complex systems of normal and abnormal development in higher organisms.

**Grant:** 2R01GM019698-33

**Program Director:** ANDERSON, JAMES J.

**Principal Investigator:** SETLOW, PETER PHD  
BIOCHEMISTRY:BIOCHEMISTR  
Y-UNSPEC

**Title:** Degradation Reactions in Spore Germination

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

**Project Period:** 1978/09/01-2008/08/31

DESCRIPTION (provided by applicant): This application proposes three major areas of work on aspects of spore formation, dormancy, resistance and germination with the bacterium *Bacillus subtilis*. These areas are: 1) determine the structure and function of the complex between alpha/beta-type small, acid-soluble spore proteins (SASP) and DNA; 2) determine the structure, mechanism and function of the protease (GPR) that degrades SASP during spore germination; and 3) investigate various aspects of spore germination. Specific aims include: a) determine the structure of SASP-DNA complexes by X-ray crystallography and use these data as well analysis of SASP with further site directed changes to determine the mechanism whereby SASP provide DNA protection; b) mutagenise the GPR coding gene to obtain variants that have lost catalytic activity but retain structure; c) determine the structure of both the active and zymogen forms of GPR; d) examine the role of another possible SASP-specific protease encoded by the *yycC* gene; e) determine the mechanism of regulation of the *gerK* operon that encodes one of the spore's nutrient germinant (Ger) receptors essential for spore germination with nutrients; f) measure the relative level of expression of the three operons (*gerA*, *gerB* and *gerK*) that encode the spore's Ger receptors; g) elucidate the requirement for lipid addition to the proteins encoded by the C-cistrons of the operons that encode the Ger receptors; h) examine the function of the SpoVA proteins in movement of dipicolinic acid (DPA) and/or ions in spore germination; i) determine the location of the SpoVA proteins in spores; j) assess the interaction of various proteins that make up Ger receptors as well as different the Ger receptors themselves; k) determine the factors that affect the spontaneous spore germination that does not require nutrients; l) isolate and characterize mutants that are altered in spontaneous spore germination and mutants whose spores germinate well in the presence of ion channel blockers; and m) express Ger receptors and SpoVA proteins in *E. coli* to study their function.

**Grant:** 2R01GM020011-33  
**Program Director:** JONES, WARREN  
**Principal Investigator:** WALSH, CHRISTOPHER T PHD  
**Title:** Modular Enzymatic Assembly Lines for Antibiotics  
**Institution:** HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA  
**Project Period:** 1987/09/30-2007/12/31

DESCRIPTION (provided by applicant): The main objectives of the proposal, detailed in specific aims 1a-d and 2a-c are to understand the molecular logic of the multimedia assembly lines for the biosynthesis of non-ribosomal peptide antibiotics. Nonribosomal peptide synthetase (NRPS) assembly lines to be analyzed include the antibiotics novobiocin and chlorobiocin, tyrocidine and gramicidin, the immunosuppressant rapamycin, the phytotoxins coronatine and syringomycin from *Pseudomonas syringae*, and the antitumor drug candidate epothilones. All these medicinally active natural products are built up as acyl chains via initiation, elongation, and termination NRPS modules. This proposal focuses on initiation (specific aim 1a-d) and termination (specific aim 2a-c) module strategies. Specific aim 1 addresses the logic, organization, and catalytic specificity of free standing A-T domain subunits acting as initiation modules in nontraditional NRP assembly. This includes the early steps that form the bicyclic aminocoumarin scaffold of novobiocin as well as the late stage reactions that generate the 5-methylpyrrolylcarboxyl moiety that interacts with the ATP site of the GyrB subunit of DNA gyrase. It also focuses on companion 2 His/Asp-Fe(II) enzymes proposed to be cyclopropanation catalyst (coronamic acid formation) or chlorination catalyst (4-chlorothreonine in syringomycin). Specific aim 2 explores the molecular logic for catalytic macrocyclization by the C-terminal domains of NRPS and hybrid polyketide/NRP assembly lines. Aim 2a deals with the range of macrolactamization by the Thioesterase (TE) domain excised from the tyrocidine synthetase assembly line, while aim 2b analyzes the cognate TE at the end of the EpoF subunit of the polyketide synthase for the 16 membered epothilone macrolide. Specific aim 2c examines the hybrid PK/NRP assembly line for rapamycin where the most C terminal domain in the RapP subunit is a Condensation (C) domain, not a TE domain, yet is thought to form the 34-membered macrolactone ring in both rapamycin and the cognate FK506 immunosuppressants. Deciphering the molecular logic of both chain initiation and chain termination strategies may facilitate subsequent efforts directed at reprogramming the initiation and termination machinery to make novel variants with altered and improved therapeutic activities.

**Grant:** 2R01GM023105-29

**Program Director:** PREUSCH, PETER C.

**Principal Investigator:** FILLINGAME, ROBERT H PHD  
BIOCHEMISTRY:BIOCHEMISTR  
Y-UNSPEC

**Title:** Proton-Translocating Sector of E coli H<sup>+</sup>-ATPase

**Institution:** UNIVERSITY OF WISCONSIN MADISON MADISON, WI

**Project Period:** 1976/05/01-2008/06/30

DESCRIPTION (provided by applicant): The H<sup>+</sup>-transporting F<sub>1</sub>F<sub>0</sub> ATP synthases of oxidative phosphorylation in mitochondria and bacteria are very similar. Rotation of subunit gamma within the core of the alpha-3-beta-3 hexamer of F<sub>1</sub> drives ATP synthesis by a unique rotary catalytic mechanism. H<sup>+</sup> transport through transmembrane F<sub>0</sub> drives rotation of an oligomeric ring of c subunits connected with gamma, and results in ATP synthesis in catalytic sites at the alpha-beta interface. A stator complex of F<sub>0</sub> subunits a and b and F<sub>1</sub> subunit delta extends from the membrane to the top of the F<sub>1</sub> molecule and holds alpha-3-beta-3 fixed, relative to the membrane, allowing the c-gamma complex to rotate within. The mechanism of coupling H<sup>+</sup> transport and c-ring rotation is poorly understood. The structure of subunit c was solved by solution NMR and the c-ring has been modeled. Biochemical evidence indicates that one of the helices of subunit c, which resides at the interface with subunit a, rotates between two different conformations. The concerted rotation of helices at the subunit a-c interface is proposed to mechanically drive the stepwise movement of the c-ring. This proposal focuses on the structure of subunit a, with the ultimate goal of defining its role in coupling H<sup>+</sup> transport to c-ring rotation. The global fold and packing of subunit a in native Escherichia coli membranes will be determined by cross link analysis. Aqueous access pathways in subunit a mediating H<sup>+</sup> transport from membrane surfaces to the H<sup>+</sup> binding site in subunit c will be defined, and the mechanism of gating H<sup>+</sup> access to the two sides of the membrane probed. Simultaneously, we will attempt to determine the solution structure of purified subunit a by NMR. Initially, the global fold of the purified protein in solution will be compared to that in the membrane using spin-labeled protein to establish appropriate solution conditions. Ultimately, we hope to define an atomic resolution structure that can be used in mechanistic studies. The ATP synthase is central to cellular function--it makes the ATP. Abnormalities in the enzyme lead to human disease. Closely related enzymes are responsible for vesicular acidification in human cells, and work by a similar rotary mechanism. The principles by which this enzyme works may provide fundamental insights into other transport problems in biology and medicine.

**Grant:** 2R01GM023719-29

**Program Director:** WOLFE, PAUL B.

**Principal Investigator:** MODRICH, PAUL L PHD  
BIOCHEMISTRY:BIOCHEMISTR  
Y-UNSPEC

**Title:** Molecular Mechanisms of DNA-Protein Interaction

**Institution:** DUKE UNIVERSITY DURHAM, NC

**Project Period:** 1977/08/01-2008/07/31

DESCRIPTION (provided by applicant): Mismatch repair plays a key role in genetic stabilization, with inactivation of the human pathway being the cause of several forms of cancer. This system has been conserved during evolution, and the E. coli methyl-directed reaction has served as the paradigm for study of the reaction in higher cells. Although bacterial mismatch repair has been reconstituted in a pure system, numerous questions remain concerning the mechanism of the reaction. This proposal addresses several of these issues. Mismatch recognition by MutS is responsible for initiation of repair, but function of the MutS ATPase is poorly understood. Using substrate binding and pre-steady-state kinetic methods, we will further clarify the nature of the interactions between the MutS nucleotide and DNA binding centers. A number of multiprotein.DNA assemblies have been implicated in mismatch repair, but the molecular nature of the MutL.MutS, MutH.MutL.MutS, and DNA helicase II.MutL.MutS complexes remains undefined. A second goal of this project is to establish the nature of these multi-protein DNA assemblies. This phase of the work will also address the significance of the specific interaction between the beta replication clamp and MutS. Since this interaction may be indicative of a special affiliation of the mismatch repair system with the replication apparatus, we will test the clamp loader and the beta clamp for potential modulatory effects on the initiation and excision steps of mismatch repair, and assess potential regulatory effects of MutS, MutL, and other repair proteins on the elongation activity of DNA polymerase III holoenzyme in response to presence of a mismatch within the primer-template. Basic features of the excision step of mismatch repair have been established, but the mechanism that underlies this bidirectional reaction is not understood. The third aim of this project is to further clarify the molecular details of this complex reaction with respect to intermediates, recycling of repair activities, and the basis of excision termination upon mismatch removal.

**Grant:** 2R01GM030717-21  
**Program Director:** TOMPKINS, LAURIE  
**Principal Investigator:** GOLDFARB, ALEXANDER PHD  
**Title:** Structure and Function of RNA Polymerases in E Coli  
**Institution:** PUBLIC HEALTH RESEARCH INSTITUTE NEWARK, NJ  
**Project Period:** 1983/03/01-2008/02/29

DESCRIPTION (provided by applicant): RNA polymerase (RNAP) is the principal enzyme of gene expression and the target for genetic regulation. The long-term objective of this research is the understanding of the function of bacterial RNAP as a molecular machine at the atomic level of resolution. Specifically, the aims are (1) to build a model of the active center that would assign function to specific amino acid residues, nucleotides and metal ions in reactions of RNA synthesis and degradation and to interpret structurally the interrelationship between these reactions; (2) to characterize conformational transitions in the ternary transcribing complex that modulate catalytic function; (3) to understand transitions in the initial transcribing complex that take place during initial buildup of the nascent transcript, the release of the initiation factor sigma and promoter clearance; and (4) to explore the plasticity of RNAP molecule through generation of aptamers so that multiple conformations of RNAP could be captured for crystallographic studies. To these ends, a series of functionally defined complexes will be generated, in which RNAP will be (a) stalled at a particular stage of the transcription process; or (b) complexed with a defined nucleic acid scaffold, or (c) frozen in a complex with a bound aptamer. The complexes will be studied using chemical nucleic acid-protein crosslinks, genetically engineered mutations in RNAP, and discriminative biochemical assays. The results will be interpreted with the aid of molecular modeling. The understanding of the basic transcription mechanisms generated in this research is a prerequisite for molecular interpretation of regulatory phenomena and the development of interventions into gene expression. In addition, knowledge gained in these studies will be helpful for rational design and screening of new inhibitors of RNAP, which has been a proven target for anti-microbial therapy.

**Grant:** 2R01GM033712-18  
**Program Director:** PREUSCH, PETER C.  
**Principal Investigator:** YAGI, TAKAO PHD BIOCHEMISTRY, OTHER  
**Title:** NADH-ubiquinone reductase of *Paracoccus denitrificans*  
**Institution:** SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA  
**Project Period:** 1984/12/01-2007/11/30

The mitochondrial proton-translocating NADH-quinone oxidoreductase (complex I) is one of the four enzyme complexes in the respiratory chain. Complex I is composed of at least 46 different subunits with a total molecular mass of approximately 1Mda. It is recognized that complex I has the most intricate structure of the membrane-bound enzyme complexes. At present, high resolution 3D structures are not available. Low resolution EM analyses indicate that complex I has an L-shaped structure consisting of a membrane domain and a peripheral domain extending to the matrix. Complex I contains one FMN and eight FeS clusters as cofactors. These cofactors are considered to be located in the peripheral domain. This enzyme pumps protons from the matrix side to the cytoplasmic side and builds an electrochemical gradient across the inner-mitochondrial membrane. Recently, it has been reported that defects of complex I are involved in many human mitochondrial diseases. Evidently, studies of complex I are important both in the basic sciences and in medical research. In contrast to mitochondria, the respiratory chain of bacteria harbors a proton-translocating NADH-quinone oxidoreductase which, although quite similar to complex I in terms of cofactors, is much simpler in structure. This bacterial equivalent of complex I is referred to as NDH-1 and is made up of only 14 unlike subunits, all of which have homologues in complex I. Because of its structural simplicity, NDH-1 is a useful model system for studying the structure and function of complex I. The overall goal of this grant application is to elucidate the structure and the mechanism of action of the NDH-1/complex I. The studies planned for this grant period are as follows. (1) Characterization of FeS clusters, determination of the amino acid residues involved in coordination of the FeS clusters, and clarification of physiological roles of the FeS clusters. (2) Identification of the conserved amino acid residues in the membrane domain subunits which are essential for structure and function of the NDH-1. (3) Determination of subunit-subunit interaction of the NDH-1. (4) Identification of the subunit(s) and the amino acid residue(s) labeled by photoaffinity analogues of inhibitors specific to the NDH-1/complex I.



**Grant:** 2R01GM034171-20  
**Program Director:** WEHRLE, JANNA P.  
**Principal Investigator:** SHORTLE, DAVID R MD  
**Title:** BIOPHYSICAL STUDIES OF FOLDING MUTANTS OF STAPH NUCLEASE  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 1982/05/01-2007/12/31

DESCRIPTION (provided by applicant): The principal objective of this project is a quantitative description of the physical chemistry that drives the amino acid sequence of staphylococcal nuclease into its three dimensional structure. Experimental studies of structure that persists when nuclease is not folded will employ NMR spectroscopy to measure long range structural features reflected in residual dipolar couplings (RDCs). Previous RDC data have demonstrated that a native-like topology" persists in denatured nuclease, even in the presence of 8 M urea after mutation of 10 large hydrophobic residues. While the argument is compelling for this conclusion, a much more quantitative understanding of the information in these couplings is needed to complete the picture of this poorly understood ensemble of many inter-converting conformations. Two strategies of data interpretation will be pursued that do not rely on single structures for representation of the ensemble average structure. To achieve the most detailed structure possible, many sets of RDCs will be collected with different alignment tensors, using electric fields or chemical modification to alter the alignment tensor. Attempts will be made in staphylococcal nuclease and three other proteins (ubiquitin, eglin C, and fyn-SH3 domain) to demonstrate a native-like topology in compact denatured states by direct correlation of dipolar couplings from the native and the denatured states. A novel strategy for predicting the structure of new protein folds, based on modeling side-chain/backbone interactions with phi/psi/chil propensities, will be pursued. Initial successes at CASP5 suggest that better sampling of the conformations of turns between helices and strands could lead to significant advances in predicting new folds at low resolution. Recently developed statistical potentials for phi/psi/chil angles and for local side-chain/side-chain interactions will be combined with torsion angle dynamics and applied to the prediction of protein structures at higher resolution, in refinement of both de novo models and homology models.

**Grant:** 2R01GM034558-20

**Program Director:** WOLFE, PAUL B.

**Principal Investigator:** MARIANS, KENNETH J PHD  
BIOCHEMISTRY:BIOCHEMISTR  
Y-UNSPEC

**Title:** Topoisomerases and Chromosome Segregation

**Institution:** SLOAN-KETTERING INSTITUTE FOR CANCER NEW YORK, NY  
RES

**Project Period:** 1984/07/01-2008/03/31

DESCRIPTION (provided by applicant): Accurate chromosome segregation is crucial to ensure that each daughter cell receives a complete copy of the genetic information. While the molecular mechanisms underpinning this process have been identified in eukaryotes, even forty years after the first model for chromosome segregation in bacteria was proposed in the replicon hypothesis, we know little about these events in prokaryotes. Recent observations from several laboratories indicate that chromosome segregation in *E. coli* and *B. subtilis* is not a passive event, but is orchestrated by forces generated by the processes of replication and transcription and managed by DNA packaging proteins. The long range goal of the studies supported by this grant has been to understand the roles in DNA metabolism of the four topoisomerases found in *E. coli*. During the previous grant period these studies led us to investigate the intersection between topoisomerase IV-catalyzed decatenation of daughter chromosomes, chromosome partition, and chromosome dynamics. We have: i) shown that the activity of Topo IV is temporally regulated in the cell as a result of independent cellular localization of the subunits of the enzyme and that this regulation is required for efficient chromosome decatenation; ii) demonstrated an interaction between Topo IV and the septal ring protein FtsK that stimulates the chromosome decatenation activity of Topo IV, thereby linking topological separation of the sister chromosomes to the cell division apparatus; and iii) uncovered the participation in chromosome segregation of SpcA, an integral inner membrane protein that interacts with MreB, the bacterial actin ancestor, potentially linking chromosome segregation to cellular infrastructure. We will proceed to use a combination of biochemical, cell biologic, and molecular genetic approaches to answer the following questions: What are the molecular mechanisms underlying temporal regulation of Topo IV activity in the cell? What is the role of the Topo IV-FtsK interaction in chromosome segregation? And, what is the role of SpcA in chromosome segregation?

**Grant:** 2R01GM035215-17  
**Program Director:** SHAPIRO, BERT I.  
**Principal Investigator:** CAFISO, DAVID S.  
**Title:** Molecular Mechanisms of Membrane Transport  
**Institution:** UNIVERSITY OF VIRGINIA CHARLOTTESVILLE CHARLOTTESVILLE, VA  
**Project Period:** 1985/09/06-2008/07/31

DESCRIPTION (provided by applicant): Active membrane transport is a critical process for normal cell metabolism, including the maintenance of ion gradients, osmotic balance, action potentials and apoptosis. The proposed work will focus on key questions regarding mechanisms of nutrient uptake in *Escherichia coli* and other Gram negative bacteria. In *E. coli*, rare nutrients are sequestered by specific outer membrane proteins that derive energy by coupling to the transperiplasmic protein TonB. These include BtuB, which is responsible for vitamin B12 transport, and FhuA, FecA and FepA, which are responsible for the transport of various forms of chelated iron. High resolution crystallographic models for each of these outer membrane proteins have been obtained; however, the molecular details of the transport machinery remain unclear. The proposed work will utilize site-directed spin labeling and EPR spectroscopy to test models for the molecular mechanisms of TonB-dependent transport in BtuB, and determine the mechanisms by which the transporter-TonB interaction is regulated. The mechanisms of transmembrane signaling resulting from substrate and colicin binding will be examined. Finally, because of the critical need for membrane protein structural biology, the backbone dynamics and structure of beta-barrel motifs, such as BtuB, will be compared in membrane and membrane mimetic systems. In addition to providing fundamental information on membrane proteins and transport, these systems are important to understand for several reasons. TonB-dependent transport provides a model for reversible and regulated protein-protein interactions, macromolecular assembly and transmembrane signal transduction. TonB-dependent transport is also unique to bacteria. Bacteria that are involved in many serious pathologies, such as meningitis, depend upon TonB transport for their success. As a result, understanding TonB transport may lead to the development of new classes of antibiotics that inhibit its function.

**Grant:** 2R01GM036296-18A1  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** LIDSTROM, MARY E PHD BACTERIOLOGY  
**Title:** Regulation of C1 Metabolism in Methylobacterium  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 1987/08/01-2008/02/29

DESCRIPTION (provided by applicant): The production, interconversion, and transfer of C1 units is an important metabolic system in all of biology. Methylobacterium are microorganisms capable of growth on C1 compounds as sole carbon and energy sources, and methylobacterium can be viewed as a specialized version of the C1 metabolism found in all organisms. A distinguishing feature of methylobacterium metabolism is the generation and consumption of formaldehyde as a central intermediate, the starting point for all of metabolism. We have gained major new insights into the pathways that consume formaldehyde in Methylobacterium extorquens AM1, and have developed a model for how the cell controls formaldehyde flux to achieve a dynamic balance of carbon and energy metabolism, avoiding formaldehyde toxicity. In this project, we will focus on the three pathways, or modules, that our working model predict are central to understanding flux of formaldehyde and energy metabolism, the H4MPT pathway, the H4F pathway, and the 3 formate dehydrogenases. We propose to begin to test our conceptual model of formaldehyde-related central metabolism in methylobacterium using a combination of biochemical, genetic, genomic, and computational approaches, focused initially on understanding how the cell responds to changes in the formaldehyde production rate. This complex system has two fundamental circuits that will be analyzed, the genetic circuit, consisting of the transcriptional and translational elements and the associated signaling components, with the output being transcripts and proteins, and the metabolic circuit, consisting of enzymes, cofactors, intermediates, and the associated signaling compounds, with the output being metabolic flux. Because of the difficulty of measuring all of the components and their characteristics, we will take a modular approach and measure outputs for each of the modules, integrating the results to create a systems-level understanding of response and resultant effects. The specific aims are: 1. Analyze the output of the genetic circuit with microarrays and proteomics. 2. Analyze the output of the metabolic circuit with enzyme assays, metabolite measurements, and direct flux measurements. 3. Integrate the results using computational models that correlate the functioning of the genetic circuit and the metabolic circuit. The result of this study will be a systems-level understanding of formaldehyde metabolism in methylobacterium. These approaches will provide a model for functional genomics at the physiological level, and will create a platform for future studies of the interaction between normal and stressed metabolism, and the mechanistic understanding of the interplay between genetic and metabolic circuits.

**Grant:** 2R01GM036718-18

**Program Director:** ANDERSON, JAMES J.

**Principal Investigator:** SONENSHEIN, ABRAHAM L PHD  
MICROBIOLOGY:MICROBIAL  
PHYSIOLOGY

**Title:** Regulation of glutamate synthesis in *Bacillus subtilis*

**Institution:** TUFTS UNIVERSITY BOSTON BOSTON, MA

**Project Period:** 1986/09/01-2008/07/31

**DESCRIPTION** (provided by applicant): The biosynthesis of glutamate lies at the intersection of carbon and nitrogen metabolism, linking the Krebs citric acid cycle to nitrogen assimilation through glutamine synthetase. In *Bacillus subtilis*, the genes for glutamate synthesis and for the pathways leading to the precursors of glutamate are tightly regulated by a host of proteins that respond to a variety of metabolic signals. The long-term goal of this project is to unravel and understand the network of genes, enzymes, and regulatory proteins that allow the cell to maintain tight control over glutamate accumulation. Building on knowledge gained from previous work, this proposal aims to focus on the roles of two of these regulatory proteins, CcpC and GltC. Two aspects of CcpC function will be investigated: interaction with the inducer, citrate, and the role of multimerization in repression. For GltC, the metabolite or protein that regulates its activity will be identified, in addition, the broad role of GltC in gene regulation and its functional interaction with other regulatory proteins will be explored. One of the Krebs cycle enzymes, aconitase, may have a second, non-enzymatic activity, perhaps as an RNA binding protein. The putative secondary activity of aconitase will be tested by seeking targets of such a function and by creating mutants that retain enzymatic activity but have lost the non-enzymatic activity. The implications of this second activity for sporulation in *B. subtilis* will receive particular attention. Since *B. subtilis* is a model organism for the gram-positive branch of the bacterial world, the knowledge gained here will be applied to a related, pathogenic species, *Listeria monocytogenes*. Thus, this proposal seeks to take advantage of the apparent conservation of regulatory proteins, gene organization and regulatory sites between *B. subtilis* and *L. monocytogenes* and thereby make rapid progress in an unexplored aspect of the life of an important pathogen.

**Grant:** 2R01GM036890-17A1  
**Program Director:** ANDERSON, RICHARD A.  
**Principal Investigator:** GOLDEN, JAMES W PHD  
**Title:** Regulation of Cyanobacterial Multicellular Development  
**Institution:** TEXAS A&M UNIVERSITY SYSTEM COLLEGE STATION, TX  
**Project Period:** 1986/08/01-2007/11/30

The regulation of development and cellular differentiation is important for all multicellular organisms. The nitrogen-fixing filamentous cyanobacterium *Anabaena* (also *Nostoc*) sp. PCC 7120 (hereafter *Anabaena*) provides an important model of multicellular microbial development and pattern formation. *Anabaena* reduces N<sub>2</sub> to ammonia in specialized terminally-differentiated cells called heterocysts. A one-dimensional developmental pattern of single heterocysts regularly spaced along filaments of photosynthetic vegetative cells is established to form a multicellular organism composed of these two interdependent cell types. This multicellular growth pattern, the distinct phylogeny of cyanobacteria, and the suspected antiquity of heterocyst development make this an important model system. Our long-term goal is to understand the regulatory network required for heterocyst development. This project is focused on cell-cell signaling and related regulatory pathways that control the initiation of development and pattern formation. An important advance was our identification of *patS*, which is required for normal pattern formation. Our results indicate that *patS* encodes a diffusible peptide inhibitor that regulates heterocyst pattern by lateral inhibition. To understand the mechanisms underlying the regulation, we will first identify the components of the PatS signaling pathway that are required for: controlling expression of the *patS* gene, mediating cell-to-cell communication, receiving the signal in target cells, and producing downstream responses. We will use several approaches to identify genes and proteins required for this regulatory network. The completed *Anabaena* genome sequence facilitates the analysis of mutants and allows the application of genomics-based techniques such as reverse genetics and expression profiling for defining a molecular phenotype of mutants. Determining the mechanism of PatS signaling will show how groups of apparently equivalent cells can be resolved to a single cell that becomes committed to differentiate into a heterocyst. Our specific objectives are to answer the following five questions. What genes and proteins are required for the production of the PatS signal, its propagation between cells along the filament, and its perception by target cells? What is the mechanism by which *patS* transcription is temporally and spatially regulated? What proteins interact with PatS? What is the final target of the PatS signaling pathway that controls the decision to differentiate? Do the temporal and spatial expression patterns of genes involved in PatS signaling support a lateral inhibition model for the mechanism controlling pattern formation?

**Grant:** 2R01GM037631-20  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** FINK, MITCHELL P.  
**Title:** Intestinal Perfusion and Permeability in Sepsis  
**Institution:** UNIVERSITY OF PITTSBURGH AT PITTSBURGH PITTSBURGH, PA  
**Project Period:** 1987/04/01-2008/06/30

DESCRIPTION (provided by applicant): The parenchymal organs that are most prominently affected in the multiple organ dysfunction syndrome (MODS) are the lungs, liver, kidneys and gut. The normal functioning of these organs depends on the establishment and maintenance of compositionally distinct compartments that are lined by sheets of epithelial cells. An essential element in this process is the formation of tight junctions (TJs) between adjacent epithelial cells. The TJ acts as a regulated semi-permeable barrier that limits the passive diffusion of solutes across the paracellular pathway between adjacent cells. Thus, the barrier function of the TJ is necessary to prevent dissipation of the concentration gradients that exist between the two compartments defined by the epithelium. The histopathology of MODS in humans is remarkably bland; massive cell death, whether due to necrosis or apoptosis, is almost certainly not the cause of MODS. Rather, the final step in the development of MODS is probably the widespread dysfunction of parenchymal cells in multiple organs as a result of the deleterious effects of a poorly controlled systemic inflammatory response. Thus, a hugely under-explored area of research can be summarized by this question: How does the inflammatory response lead to parenchymal cell dysfunction? Based on our work during the previous cycle of funding, we hypothesize that MODS results, at least in part, from nitric oxide (NO)- dependent perturbations in the expression and subcellular localization of TJ proteins. To test this hypothesis, we propose to study inflammation-induced alterations in epithelial barrier function and TJ formation at levels of integration ranging from whole animals to cultured cells or subcellular fractions. In vitro, we will focus on changes in intestinal epithelial permeability using Caco-2 (human enterocyte-like) monolayers as a reductionist model system. In vivo, however, in studies using mice and rats, we will evaluate changes in epithelial barrier function not only in the gut, but also in the liver and lung as well. In a series of 6 Specific Aims, we will test these Specific Hypotheses: 1) sepsis in mice leads to alterations in TJ structure and function via mechanisms that depend on the formation of NO., reactive oxygen species (ROS), and/or ONOO-; 2) the structure of epithelial TJs is deranged in patients dying with MODS; 3) decreased transcription of the TJ protein, ZO-1, is a critical step leading to inflammation- or NO-induced alterations in epithelial barrier function; 4) cytokine- or NO-induced events impair the proper packaging and targeting of the key TJ proteins, claudin-1 and occludin, in cultured Caco-2 cells; 5) post-translational modification of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) contributes to epithelial barrier dysfunction; 6) alterations in the function of Na<sup>+</sup>,K<sup>+</sup>-ATPase contribute to inflammation-induced derangements in epithelial barrier function.

**Grant:** 2R01GM037704-16A1

**Program Director:** SOMERS, SCOTT D.

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

**Title:** EPCR, TAFI as Regulators of PMN/Endothelial Interaction

**Institution:** OKLAHOMA MEDICAL RESEARCH OKLAHOMA CITY, OK  
FOUNDATION

**Project Period:** 1986/12/01-2008/08/31

DESCRIPTION (provided by applicant): These studies focus on the role of endothelial protein C receptor (EPCR) and thrombin activatable fibrinolytic inhibitor (TAFI) as regulators of the neutrophil/endothelial interaction induced by E. coli. All the inflammatory and hemostatic events studied in the baboon model of E. coli sepsis culminate in an aberrant neutrophil/endothelial interaction leading to increased permeability and coagulation disorders. EPCR and thrombomodulin (TM) are at the point of attack and therefore are both targets and regulators of this interaction through activation of protein C and TAFI by the TM/thrombin complex and through release of soluble EPCR by endothelial-derived metalloproteases. We postulate that TAFIa (procarboxypeptidase beta) attenuates neutrophil activation by inactivating C5a, and that soluble EPCR attenuates subsequent tight binding of neutrophils to endothelium. The close association of EPCR and TAFIa with the endothelium and neutrophils favor these actions. Two general questions are: What is the response and distribution of EPCR and TAFI between endothelium and neutrophils? Can the information be used to design and time intervention using soluble EPCR and TAFIa that would improve the efficacy of activated protein C? To study such questions we have adopted the sublethal model of E. coli sepsis, because the otherwise lethal events are stretched out over time into an initial host (stage 1) and ischemia reperfusion (stage 2) responses. This model allows one to track and intervene with the responses of these regulatory components at critical points of the response to E. coli. Specific questions include what are the timing and distribution of EPCR and TAFI between endothelium and neutrophils with respect to mediators (e.g., C5a) and adherence of neutrophils to the microvascular endothelium? Are there critical events involving these regulators in stage 1 that determine subsequent stage 2 events and whether the response becomes lethal? Can APC and either sEPCR or TAFI be used together to better regulate the neutrophil/endothelial response to E. coli? How critical is timing of intervention in determining whether it is beneficial or harmful? Changes in the expression and distribution of EPCR, TAFI, protein C, thrombomodulin, tissue factor (etc.) on the endothelium, perivascular tissues and neutrophils will be assessed using immunohistochemical and confocal imaging techniques. This includes FACS analysis, and determination of neutrophil half-life. The responses of plasma factors will be followed with ELISAs. Standard physiological parameters will be followed (e.g., temperature, CBC, blood pressure and global assays of hemostatic function)



**Grant:** 2R01GM038032-18  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** LANG, CHARLES H.  
**Title:** Cytokine Regulation of IGF System During Infection  
**Institution:** PENNSYLVANIA STATE UNIV HERSHEY MED HERSHEY, PA  
CTR  
**Project Period:** 1987/04/01-2008/06/30

**DESCRIPTION** (provided by applicant): Muscle wasting remains a major cause of morbidity and mortality in patients after injury and infection. Although the sepsis-induced decrease in lean body mass is undoubtedly multifactorial, our previous work demonstrates a causal relationship between the insulin-like growth factor (IGF) system, tumor necrosis factor (TNF)- $\alpha$ , and translational control of muscle protein synthesis. Moreover, the sepsis-induced changes appear unique to skeletal muscle. The working hypothesis is tested by the proposed research is that the sepsis induced decrease in the local concentrations of IGF-1, regulated by the overproduction of inflammatory cytokines, impairs muscle protein synthesis via disruption of the mTOR (mammalian target of rapamycin) signaling complex. A tripartite strategy is used whereby studies are proposed to examine a) the novel mechanism by which sepsis produces growth hormone (GH) resistance in skeletal muscle and decreases synthesis of IGF-1; b) the mechanism by which sepsis and TNF $\alpha$  regulate translational control of muscle protein synthesis and thereby influence the anabolic effects of GH, IGF-1 and nutrient signaling; and c) the autocrine/paracrine role of inflammatory cytokines in regulating muscle IGF-I synthesis and protein synthesis. The proposed research has the following specific aims: (1) Elucidate the mechanism by which sepsis produces GH resistance in muscle by impairing Stat5 transcriptional activity, thus uncoupling Stat5 phosphorylation from IGF-I synthesis. (2) Determine whether inflammatory cytokines alter the regulation of IGF-I promoter activity by GH. (3) Delineate the mechanism by which sepsis and TNF $\alpha$  modulate mTOR-dependent signaling in muscle and thereby impair protein synthesis and translational initiation. (4) Determine whether the enhanced synthesis of TNF $\alpha$  impairs GH signaling and IGF-I expression in skeletal muscle in response to sepsis via a systemic and/or local cytokine network. (5) Determine whether sepsis alters mTOR signaling via a systemic and/or local muscle cytokine network. The ability to perform complementary studies using both rats and transgenic mice as well as cultured myocytes and isolated muscle cells places us in a unique position to delineate the molecular mechanisms responsible for the sepsis-induced impairment in muscle protein synthesis. These data will provide a better understanding of the numerous factors influencing the IGF system and cellular metabolism, which is needed to both realize the full potential and avoid possible pitfalls of anabolic agents used in the management of critically ill patients.

**Grant:** 2R01GM040287-13A1  
**Program Director:** SCHWAB, JOHN M.  
**Principal Investigator:** LIPSHUTZ, BRUCE H  
**Title:** Metal-Mediated Routes to Biaryls  
**Institution:** UNIVERSITY OF CALIFORNIA SANTA BARBARA SANTA BARBARA, CA  
**Project Period:** 1991/01/01-2008/03/31

DESCRIPTION (provided by applicant): Biaryls represent a major area of natural and unnatural products chemistry. Given the widespread occurrence of physiologically active compounds in Nature that contain a biaryl axis, many of which due to hindered rotation possess an element of axial chirality, methodology is needed to respond to these special synthetic challenges. Representative targets which highlight existing limitations yet which provide opportunities for significant advances in this area include the clinically essential antibiotic vancomycin, and the potent anti-AIDS biaryls, the michellamines. Using a judiciously placed internal phosphine ligand in an aryl halide coupling partner, the directionality associated with our key Suzuki-biaryl coupling-based approach to the vancomycin biaryl and the subunits of the michellamines will be controlled. Alternatively, a conceptually new entry to stereocontrolled biaryls, as applied to vancomycin, will be pursued using a Bergman cyclization of a substituted nonracemic endiyne. The chemistry of biaryl constructions, which is usually effected in solution using Pd(0) catalysis, is to be pursued via an alternative metal system: nickel. Proposed herein are new methods for heterogeneous catalysis based on Ni/C, to be examined under microwave conditions, and the next generation species nickel-on-graphite ("Ni/Cg"), which appears to offer a different reactivity profile. Finally, a new series of nonracemic ligands based on the binaphthyl core, in particular of NOBIN, will be constructed. The approach presented will provide entry to unprecedented substitution patterns on this ligand system, as well as opportunities for their mounting on a solid support for use, and re-use, under heterogeneous conditions. A particular, albeit representative, application of a novel substituted cyclo-NOBIN will be studied for selected asymmetric aldol reactions.

**Grant:** 2R01GM041934-14  
**Program Director:** WOLFE, PAUL B.  
**Principal Investigator:** GROSSMAN, ALAN D PHD  
**Title:** Cell Cycle and sporulation in *Bacillus subtilis*  
**Institution:** MASSACHUSETTS INSTITUTE OF CAMBRIDGE, MA  
TECHNOLOGY  
**Project Period:** 1989/07/01-2007/11/30

Our long term goals are to understand the bacterial cell cycle and the connections among chromosome replication, organization, partitioning, and gene expression. Cell growth, propagation, and development all require the duplication and faithful segregation of chromosomal DNA. To ensure that these essential processes are proceeding normally, cells possess regulatory mechanisms that couple division or development to the fidelity of chromosomal transmission. Many diseases, including cancers, result from aberrant regulation of the cell cycle and loss of fidelity of chromosome transmission. In addition, microbial pathogenesis often depends on normal bacterial growth in the host. This proposal focuses on several aspects of the *Bacillus subtilis* cell cycle with three areas of particular interest related to chromosome dynamics and gene expression: 1) the regulatory response to arrest of replication forks; 2) initiation of replication and the subcellular positioning of *oriC* and the replisome; and 3) proteins and DNA sites involved in chromosome compaction and cohesion and their effects on gene expression. We will use a variety of approaches and methodologies to characterize: genes controlled in response to replication fork arrest; the role of DnaA, the replication initiator protein, in the transcriptional response to replication fork arrest; the subcellular positioning of the chromosomal origin of replication and its association with the membrane; the functions of two essential genes replication initiation genes; factors controlling replisome positioning in the cell; proteins involved in chromosome compaction and cohesion, and their roles in gene expression. The fundamental principles and mechanisms controlling these processes are easily studied in *B. subtilis* using a combination of cell biological, genetic, molecular, physiological, biochemical, and bioinformatic approaches. Because many of the proteins involved in these processes are highly conserved, insights gained from work with *B. subtilis* are likely to provide information regarding similar processes in a wide variety of organisms. Learning more about the essential mechanisms governing the chromosome replication and partitioning could lead to the identification of targets for the development of new antibiotics.

**Grant:** 2R01GM042577-12  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** GAMELLI, RICHARD L  
**Title:** Myelopoietic Stimulation Following Burn Injury  
**Institution:** LOYOLA UNIVERSITY CHICAGO MAYWOOD, IL  
**Project Period:** 1989/08/01-2007/11/30

Critical injury and sepsis continues to be a major health concern in the US. Many of these patients succumb to infections that lead to unabated systemic inflammatory response and multiple organ failure. Burn trauma is associated with loss of the barrier and immune protection afforded by the skin. As a consequence, severe disturbances in physiological, metabolic, nutritional and immunological parameters ensue. One of the sentinel cellular features of critical injury and sepsis is the dysregulation of leukocyte function. Our group has demonstrated a significant role for bone marrow myelopoiesis (a developmental program that is responsible for the continuous production of many leukocytes) in the pathophysiology of burn injury and sepsis. In the last funding period, we have been able to demonstrate that burn injury and sepsis enhance bone marrow monocytopoiesis through upregulation of M-CSF receptors while causing granulocytopoietic arrest through a down regulation in G-CSF receptor expression. During that period we have also identified mediators, prostaglandin E2 (PGE2) and granulocyte colony stimulating factor (G-CSF) that play a significant role in the myelopoietic alterations of burn injury. We have also demonstrated that the thermal injury and sepsis-mediated alterations in monocyte development in the bone marrow and the function of progenitor derived macrophages (PDMo) are regulated by PGE2, G-CSF and by the severity of burn injury and sepsis. The responses of PDMo are similar to the responses of peritoneal macrophages (PMo) emphasizing the relevance of PDMo to the pathobiology of injury and sepsis. Building on our findings during the previous funding, we propose to study the regulation of bone marrow monocyte development and function by the micro-environmental changes imposed by thermal injury and sepsis. We will test this premise in our established murine model of burn injury and sepsis at clinically relevant times following thermal injury and sepsis. In the first aim we will establish that functional phenotype of monocytes and macrophages in burn injury and sepsis is initiated and set in motion during monocyte development within the bone marrow. Changes in cytokine responses, phagocytosis, and antigen presentation are some of the cellular functions that will be documented. Since monocytopoiesis is enhanced in the presence of elevated G-CSF levels in burn injury and sepsis, second aim will establish how G-CSF may modulate monocyte development, function and hematopoietic gene expression patterns. In the last aim, we will study the capacity of G-CSF and PGE2 to modulate monocyte progenitor differentiation into macrophages and their ability to induce genotypic and phenotypic changes. Completion of these aims will provide critical information on mechanisms underlying the observed macrophage phenotypic heterogeneity seen under injury conditions and allow us to appropriately formulate, and test new therapies against sepsis.

**Grant:** 2R01GM043577-17A1  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** PIGGOT, PATRICK J PHD BIOCHEMISTRY:NUCLEIC  
ACID  
**Title:** GENE EXPRESSION DURING SPORULATION  
**Institution:** TEMPLE UNIVERSITY PHILADELPHIA, PA  
**Project Period:** 1986/07/01-2007/12/31

DESCRIPTION (provided by applicant): Cell differentiation is a fundamental biological process. Central to it are the establishment of distinct programs of gene expression in the different cell types and the coordination of gene expression with morphological change. Formation of spores by *Bacillus subtilis* is a primitive system of cell differentiation that has become a paradigm for the study of cell differentiation in prokaryotes because of the ease of its genetic manipulation. All the key regulators of spore formation are also identified in all sequenced species of Bacillaceae, including the pathogens *Bacillus anthracis* and *Clostridium difficile*. Sporulation involves a characteristic division into two distinct cell types, the mother cell and the prespore. The prespore is engulfed by the mother cell and develops into the mature, resistant spore. Sporulation requires the action of four RNA polymerase sigma factors, sigmaF and then sigmaG in the prespore and sigmaE and then in sigmaK the mother cell. The major objectives here are to understand how compartmentalized gene expression is established and maintained and how gene expression is coordinated with morphological change. Most of the proposal centers on *B. subtilis*. A series of interconnected lines of research will be pursued. It is proposed to investigate why sigmaG activity switches from the prespore to the mother cell in *spolAdelta* mutants. It is proposed to identify and characterize division genes and regulators of sigmaF activation that are required for prespore-specific expression using a two-part compartmentalization test we have developed. It is proposed to investigate why certain sigmaF-directed genes are poorly expressed when they are relocated near the chromosome terminus. It is proposed to identify and characterize genes involved in temporal control of sigmaF and sigmaG activity. It is proposed to investigate the establishment of compartmentalization in *Sporosarcina ureae* where the sporulation division is medially located, in contrast to its grossly asymmetric location for species of *Bacillus* and *Clostridium*.

**Grant:** 2R01GM047112-32

**Program Director:** IKEDA, RICHARD A.

**Principal Investigator:** SWITZER, ROBERT L  
PHD  
BIOCHEMISTRY:BIOCHEMISTR  
Y-UNSPEC

**Title:** NOVEL CONTROL MECHANISMS IN ENDOSPORE FORMATION

**Institution:** UNIVERSITY OF ILLINOIS URBANA- CHAMPAIGN, IL  
CHAMPAIGN

**Project Period:** 1991/05/01-2007/03/31

DESCRIPTION (provided by applicant): This research will study the regulation of bacterial genes by an important class of mechanisms called attenuation or antitermination. Such mechanisms control gene expression by regulating transcription termination signals that lie between the start of transcription (mRNA synthesis) and the coding regions of the genes being regulated. They are very widespread in bacteria. Two such systems have been discovered in the Switzer laboratory; these will be biochemically characterized in detail. In the first system the attenuation regulatory protein PyrR regulates pyrimidine biosynthetic (pyr) genes by binding to specific sites on pyr mRNA. A detailed study of PyrR-RNA interaction will be undertaken by binding studies using surface plasmon resonance and by high-resolution x-ray crystallography of PyrR-RNA complexes. The role of transcriptional pausing in PyrR action will be studied by genetic methods. The integrated regulation of the *B. subtilis* pyr operon by PyrR at three termination sites in the operon will be characterized by quantitative measurements of pyr RNA species in vivo. In the second system direct regulation of termination in the 5' leader of *B. subtilis* pyrG RNA (encoding CTP synthetase) by CTP without involvement of a regulatory protein has been demonstrated. The mechanism of this regulation will be studied by biochemical analysis of pyrG transcription in vitro. Comparative genomics demonstrates that the regulation of pyr genes by PyrR-dependent processes and pyrG antitermination similar to *B. subtilis* are found in many diverse bacterial genera, including many disease-causing bacteria in which antibiotic resistance is a growing clinical problem and others that are important in fermentation and biotechnology. A final objective of this research is to characterize the regulation of pyr genes in *Mycobacteria*, in which PyrR appears to act as an inhibitor of protein synthesis. It is planned to use nonpathogenic species for these investigations, but *Mycobacteria* are the agents of tuberculosis and leprosy.

**Grant:** 2R01GM051610-08A1

**Program Director:** SHAPIRO, BERT I.

**Principal Investigator:** PARK, JAMES T PHD  
BIOCHEMISTRY:BIOCHEMISTR  
Y-UNSPEC

**Title:** Muropeptide Recycling Pathway & Beta Lactamase Induction

**Institution:** TUFTS UNIVERSITY BOSTON BOSTON, MA

**Project Period:** 1996/05/01-2007/08/31

DESCRIPTION (provided by applicant): Amazingly, *Escherichia coli* degrades over 60% of the murein (peptidoglycan) from its sidewalls each generation and is able to reutilize the components. This process is known as recycling. The goal of this research is to identify and characterize all the enzymes used by *E. coli* to reutilize cell wall peptidoglycan components. Recycling involves over 10 enzymes that appear to serve no other purpose than to facilitate breakdown and reutilization of murein components. Several outstanding questions remain to be answered in order to have a full understanding of the process. The recycling pathway is required for beta-lactamase induction in many Gram negative organisms and, in addition, we have recently observed that mutations in certain recycling genes result in autoaggregation and biofilm formation. Thus the pathway is of interest in the health related areas of resistance to penicillins and biofilm formation. Specific aims: Aim #1: Determine the pathway for utilization of N-acetylglucosamine in the absence of the N-acetylglucosamine kinase. We have already achieved the original goal of Aim 1, namely, Identify the kinase gene and demonstrate its role in reutilization of N-acetylglucosamine. Aim #2: Determine how *E. coli* degrades anhydro-N-acetylmuramic acid. Our recent results indicate that a kinase and an etherase may be involved. Hence our current efforts are aimed at purifying these activities in order to identify the genes responsible for the activities. Aim #3: Determine the true inducer of beta-lactamase. Aim #4: Determine how expression of antigen 43 is related to the recycling of murein tripeptide. Antigen 43 causes autoaggregation which facilitates biofilm formation. Deletion of either murein peptide ligase (mpl) or murein peptide amidase A (mpaA) results in production of tripeptide and antigen 43 suggesting that accumulation of the murein tripeptide, L-Ala-gamma-D-Glu-meso-diaminopimelic acid, may be involved.

**Grant:** 2R01GM051986-10  
**Program Director:** ZATZ, MARION M.  
**Principal Investigator:** BRUN, YVES V  
**Title:** Global control of differentiation in *Caulobacter*  
**Institution:** INDIANA UNIVERSITY BLOOMINGTON BLOOMINGTON, IN  
**Project Period:** 1995/01/01-2007/12/31

The correct execution of a developmental program requires that individual events proceed in an orderly fashion. Differentiating cells have to integrate processes such as DNA replication, cell division, and changes in morphology. The long-term goal of this project is to understand how cell division and cell differentiation are regulated and integrated in the bacterium *Caulobacter crescentus*. Each cell division is asymmetric and produces two different cell types: a motile swarmer cell and a sessile stalked cell. Only the stalked cell is competent to replicate DNA and divide. The asymmetric predivisional cell has a flagellum at one pole and a stalk at the opposite pole. The establishment of asymmetry prior to cell division is tightly coupled to cell cycle progression by DNA replication and cell division checkpoints. The proposed research has three main objectives. The first objective is to identify the mechanisms responsible for the cell cycle control of cell division. One checkpoint that couples cell division to DNA replication is mediated by the master cell cycle response regulator CtrA. Experiments are proposed to determine how the activity of CtrA is regulated by DNA replication and how the cell sets the stage for the replication checkpoint by degrading cell division proteins at the end of every cell cycle. The second objective is to define the genes and the cell division checkpoint mechanism that couple polar development to cell division. The sigma-54 specific response regulator, TacA, is required for the cell division checkpoint and its mechanism of action will be determined. The TacA-dependent gene(s) involved in checkpoint control will be identified and studied, and the mechanism by which cell division inhibition is transduced to TacA will be determined. The third objective is to investigate the function of the polar organelle development protein, PodJ, in regulating pili and holdfast synthesis, two events that are blocked by the cell division checkpoint. The role of PodJ in the localization of critical regulators of development will be determined and the mechanism of PodJ proteolytic processing, which is coupled to cell division, will be investigated. These studies will lead to a better understanding of the mechanisms that regulate cell differentiation.



**Grant:** 2R01GM052021-10  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** GELLER, DAVID A MD  
**Title:** Regulation of the Human iNOS Gene in Sepsis and Trauma  
**Institution:** UNIVERSITY OF PITTSBURGH AT PITTSBURGH PITTSBURGH, PA  
**Project Period:** 1995/06/01-2008/06/30

**DESCRIPTION** (provided by applicant): The human inducible nitric oxide synthase (hiNOS) gene is expressed in nearly every organ during sepsis and other inflammatory conditions. While NO synthesis has beneficial effects during acute inflammation, excessive NO production is harmful. Chronic hiNOS expression has been implicated in NO-mediated tissue damage leading to diabetes, neurodegenerative disorders, and certain cancers. Our laboratory has cloned the human iNOS gene from cytokine-stimulated hepatocytes, and we have shown that cytokine-responsive DNA elements are located approximately 5 kb upstream in the promoter region. We found that TNF $\alpha$  and IL-1 $\beta$  signal through NF-kappaB, while IFN $\gamma$  signals through Stat-1 by binding to cis-acting elements at -5.2 and -5.8 kb in the promoter, thereby providing a molecular basis for cytokine synergy. Further functional roles have been demonstrated for AP-1, C/EBP $\beta$  (LAP), and KLF6. Importantly, we have identified mechanisms for hiNOS repression that involve NF-kappaB repressing factor (NRF), LIP, and p53 proteins. A subsequent chromatin structure analysis using DNase I mapping and in vivo footprinting revealed that regulation of hiNOS transcription was even more complex than originally anticipated and exhibited tissue-specific control by both basal and inducible transcription factors. Most recently, we have identified a novel role for the Wnt beta-catenin/Tcf-4 signaling pathway in regulating hiNOS expression. Therefore, our hypothesis is that the regulation of hiNOS gene expression requires an orchestrated flow of positive and negative transcription factors binding to a cis-acting upstream enhancer region located between -5.0 and -7.0 kb in the hiNOS promoter. In addition, a crucial downstream promoter region has been identified at -0.2 kb that is permissive for cytokine-induced transcription. In this proposal, we will pursue two specific aims to further elucidate the molecular mechanisms involved: **AIM I: TO DEFINE THE TRANSCRIPTION FACTORS AND FUNCTIONAL PROMOTER ELEMENTS RESPONSIBLE FOR CYTOKINE INDUCTION OF THE HUMAN iNOS GENE.** ChIP assay will be used to confirm in vivo protein-DNA interactions for NF-kappaB, Stat-1, and AP-1 in the upstream enhancer region. New roles for Ets-1 and Oct-1 will be tested by gel shift and promoter transfection studies. Protein-protein interactions between NRF and NF-kB will be pursued, as well as mechanisms of downstream control elicited by LAP/LIP, KLF6, and p53. **AIM II: TO DETERMINE THE ROLE OF THE WNT Beta-CATENIN/TCF-4 SIGNALING PATHWAY IN REGULATING HUMAN iNOS EXPRESSION.** A functional role for Beta-catenin/Tcf-4 binding to two TBE sites will be determined, as well as possible interactions with other transcription factors governing hiNOS expression. The information gained will increase our understanding of the control of hiNOS transcription, describe novel mechanisms of cytokine-synergy in signal transduction, and help in designing therapeutic strategies for pathophysiologic disease states where hiNOS expression is relevant.

**Grant:** 2R01GM052465-09  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** FARRAND, STEPHEN K  
**Title:** CELL-CELL SIGNALING IN MICROBE-HOST INTERACTIONS  
**Institution:** UNIVERSITY OF ILLINOIS URBANA- CHAMPAIGN, IL  
CHAMPAIGN  
**Project Period:** 1995/05/01-2008/07/31

**DESCRIPTION** (provided by applicant): Interactions between microbes and their hosts, including man, depend on specific communication systems. Thus bacteria, including pathogens, perceive their hosts by sensing chemical signals and respond appropriately. Understanding these signaling pathways, and how the microbe and its host respond, could lead to strategies for preventing pathogenesis or fostering beneficial relationships. *Agrobacterium tumefaciens*, which interacts with plants, provides an excellent model for such studies. As part of this interaction, the bacterium responds to a plant signal by eliciting a second signal that is then perceived by the entire bacterial population. This second quorum-sensing signal controls transfer of the Ti plasmid, a virulence element, to other bacteria. The long term goal is to understand this hierarchical signaling process including how the quorum-sensing signal, called AAI triggers activation of the positive transcription factor, TraR, how TraR retains its activity, and how a specific antiactivator, TraM, interferes with TraR activity. There are three goals for the project period. First we will examine the effect of signal loss on the structure of TraR using genetic screens and biochemical and spectral technologies. We will probe the structure of monomer TraR using a mutant that can bind signal but cannot form dimers. In the second goal we will examine the interaction between TraR and components of RNA polymerase including RpoA and RpoD. Our goal is to identify the amino acids of TraR, of RpoA, and of RpoD that contribute to stable complexes. We will establish conditions for isolating stable complexes of TraR and a C-terminal fragment of RpoD preparatory to efforts to determine the crystal structure of the complexes. In the second goal, we will assess the nature of the interaction between TraR and the antiactivator TraM. We have established a collaboration to determine the crystal structure of TraM and also the crystal structure of the complex formed by TraM and TraR. We also will probe the role of TraM and Lon protease in determining the stability of TraR using physiological tests to measure rates of TraR turnover. We also will establish an in vitro system using purified proteins to characterize the Lon-mediated degradation of TraM. In the third goal we propose to explore the evolutionary diversity of the Ti plasmid quorum-sensing systems by isolating and characterizing plasmids that induce transfer genes in response to novel plant signals.

**Grant:** 2R01GM053818-22  
**Program Director:** JONES, WARREN  
**Principal Investigator:** PARRY, RONALD J. PHD CHEMISTRY:ORGANIC  
**Title:** Biosynthesis of Some Microbial Metabolites  
**Institution:** RICE UNIVERSITY HOUSTON, TX  
**Project Period:** 1978/09/01-2007/11/30

Investigations of the biosynthesis of two novel microbial metabolites will be continued. The first is the antitumor agent valanimycin, an azoxy compound produced by *Streptomyces viridifaciens*. As a naturally occurring azoxy compound, valanimycin is a member of a growing family of natural products that includes substances with antitumor, antifungal, and carcinogenic activity. Previous investigations have led to the elucidation of the early steps in the valanimycin biosynthetic pathway and, more recently, to the cloning and analysis of the valanimycin biosynthetic gene cluster. The long term objective of future studies of valanimycin is to identify and investigate the proteins encoded by the gene cluster that are responsible for the formation of the azoxy group of valanimycin, and thereby to elucidate the biochemical mechanism of azoxy group formation. These studies are significant because they should help illuminate the chemistry of N-N bond formation associated with the biosynthesis of other known bioactive natural products that contain N-N bonds. Future studies of valanimycin have several specific goals. The first goal is to investigate the role played by VImL, an apparent seryl-tRNA synthetase, in valanimycin biosynthesis, including an investigation of the possibility that a seryl-tRNA is an intermediate in the valanimycin biosynthetic pathway. The second goal will be to overproduce and investigate the function of several other proteins in the gene cluster that may be involved in the processing of the known intermediate isobutylhydroxylamine and in the processing of a serine derivative such as seryl adenylate or a seryl-tRNA. The third goal will be to create non-polar disruptions in genes of unknown function in the gene cluster and analyze the chemical phenotype of the resulting mutants to detect new intermediates in the pathway. The second metabolite to be investigated is the antitumor agent sparsomycin, a potent inhibitor of protein biosynthesis produced by *S. sparsogenes*. Sparsomycin is an important target for biosynthetic investigation because it is known to inhibit the peptidyltransferase step in protein biosynthesis and because previous studies have shown that sparsomycin biosynthesis involves novel biochemistry. Future investigations of sparsomycin will focus on cloning the sparsomycin resistance gene from *S. sparsogenes* to understand the mechanism of self-resistance in this organism and to gain access to the biosynthetic genes.

**Grant:** 2R01GM054060-09  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** GOLENBOCK, DOUGLAS T  
**Title:** Phagocyte Receptors for Lipid A  
**Institution:** UNIV OF MASSACHUSETTS MED SCH WORCESTER, MA  
WORCESTER  
**Project Period:** 1997/01/01-2008/03/31

**DESCRIPTION** (provided by applicant): Gram-negative bacterial septicemia remains an important cause of morbidity and mortality in the United States. Gram-negative sepsis begins when bacterial membranes shed endotoxin (lipopolysaccharide, LPS) and engage signaling receptors on the surface of phagocytes and other LPS-sensitive cells. Although additional receptor components may yet be discovered, the names of the basic components of the LPS response machinery are now known. These include LPS-binding protein (LBP), CD14, Toll-like receptor (TLR) 4, MD-2 and one or more "Toll, interleukin 1, resistance" (TIR)-domain containing adapter proteins. The hypothesis to be tested represents the current dogma: sepsis begins with LBP-mediated presentation of LPS to CD14. CD14 presents LPS to the TLR4/MD-2 complex. LPS binding to TLR4 results in receptor dimerization and recruitment of MyD88/Mal complexes resulting in the initiation of the NF- $\kappa$ B and IRF signal transduction pathways. These transcription factors drive cytokine production, resulting in septicemia. Many aspects of this dogma need considerable reassessment and refinement. For example, while preliminary data confirm that LPS directly binds to MD-2, and suggest that TLR4 is also bound, TLR4/MD-2 forms large multimers- much larger than dimers- after being bound by LPS. In addition to MyD88 and Mal/TIRAP, at least two other adapter molecules, TRAM (which we recently discovered) and TRIF, are involved in the TLR4 pathway. We propose 4 specific aims: 1) To characterize the binding of LPS to TLR4 and MD-2. 2) To determine if the TLR4/MD-2 complex activates signal transduction by creating 'signalosome' clusters. The aim is meant to precisely quantify receptor size and composition of the signalosome in LPS-stimulated cells. 3) To assess the roles of the five TIR domain containing adapter molecules in TLR4 signal transduction. This aim combines aspects of biochemistry, molecular genetics and confocal microscopy to define the role of adapters in LPS stimulation. And finally, 4) to characterize the role of TRAM, a newly discovered adapter molecule, in LPS signal transduction by generating and characterizing a mouse with a targeted deletion in TRAM. Cells from this mouse will be tested for responses to LPS and other microbial products. In addition, we will challenge this knockout animal with Salmonella to determine if and how TRAM expression contributes to host defense.

**Grant:** 2R01GM054365-22  
**Program Director:** RODEWALD, RICHARD D.  
**Principal Investigator:** ORDAL, GEORGE W.  
**Title:** CHEMOTACTIC SENSORY TRANSDUCTION IN BACILLUS SUBTILIS  
**Institution:** UNIVERSITY OF ILLINOIS URBANA- CHAMPAIGN, IL  
CHAMPAIGN  
**Project Period:** 1983/08/01-2008/05/31

Our goal during the past thirty years of NIH funding has been to delineate the mechanism of chemotaxis in the Gram-positive bacterium *Bacillus subtilis*. During the past decade, the realization has emerged that the *B. subtilis* mechanism might be the ideal paradigm for understanding the mechanism in the broad sweep of Bacteria and Archaea since it is so similar to that in the Archaea. It is an ancient mechanism and close to the progenitor mechanism that existed just before the separation of the Bacteria and Archaea. Thus in studying this mechanism, we can see where the current mechanisms in Bacteria and Archaea came from and get insights into how they might currently work. The mechanism involves two proteins, found in the Archaea but not in *Escherichia coli* CheC and CheD. It also involves receptors that, like those in the Archaea, have two pairs of insertions of four turns of  $\alpha$ -helix in two locations in the cytoplasmic region of the receptors, compared with what is found in *E. coli*. It also involves another protein CheV, found only in Bacteria but very widespread there, although not in *E. coli*. The *B. subtilis* mechanism has a large switch protein, FliY, whose C-terminal region is homologous to the small *E. coli* protein FliN but no one knew why the protein was so large. During the last funding period biochemical events associated with each of the novel proteins or with the receptor differences between *B. subtilis* and *E. coli* have been described so that the usefulness of the *B. subtilis* mechanism as a paradigm has become much enhanced. CheC and FliY are CheY-P phosphatases, CheD is a deamidase and stimulates CheC, whereas CheC inhibits CheD. CheV brings about adaptation by becoming phosphorylated. Methylation of receptors at nearby sites has opposite effects on the kinase. At this point, we wish to take our quest to understand this mechanism to a new level, namely, to refine our work on individual proteins and to understand how the various chemotaxis proteins interact to bring about excitation and adaptation--that is, chemotaxis. We have developed the genetic stocks and in vitro assays to do this. We propose to map out where each of the proteins interacts with the others and, using mutants, show the function of each interaction. We propose detailed characterization of receptor methylation to pin down the roles of each site. We propose experiments to clarify the roles of each of the enzymatic and binding functions of CheC, CheD, and CheV.

**Grant:** 2R01GM054395-22

**Program Director:** ANDERSON, JAMES J.

**Principal Investigator:** MORAN, CHARLES P PHD GENETICS:GENETICS-OTHER

**Title:** RNA Polymerase and Bacterial Differentiation

**Institution:** EMORY UNIVERSITY ATLANTA, GA

**Project Period:** 1996/06/01-2008/08/31

DESCRIPTION (provided by applicant): As the bacterium *Bacillus subtilis* differentiates from the vegetative form into a dormant endospore, complex morphological and physiological changes occur that require the expression of many genes. During the process, four new RNA polymerase sigma subunits appear displacing one another and conferring on the RNA polymerase different specificities for the recognition of different classes of promoters. These sigma factors appear in the order sigmaF, sigmaE, sigmaG, and sigmaK. In addition, the action of RNA polymerase on specific promoters is controlled by DNA binding proteins to affect both temporal and cell-type specific transcription. The sporulating bacterium is divided into two compartments (forespore and mothercell) within which different sets of genes are expressed. In addition to the sporulation-specific induction of sigma factor synthesis, the activity of each sigma factor is regulated. Regulation of sigma factor activity is used to synchronize the different programs of gene expression in the two cellular compartments during development. The mechanisms that coordinate sigma factor activities in the forespore and mothercell are not completely understood, but appear to involve targeting of proteins to specific subcellular locations where they provide the conduits for intercellular signaling. We will investigate the mechanisms of promoter activation by Spo0A, a DNA binding protein, to understand how it interacts with two different sigma factors to affect both temporal and cell-type specific activation of promoters. We will also investigate the mechanisms involved in the intercellular signaling between sigmaE in the mothercell and sigmaG in the forespore, especially the mechanisms that target the signaling proteins and others made in the mothercell to the forespore. Elucidation of the mechanisms that control the temporal expression of genes, and synchronize developmental programs of gene expression in *Bacillus subtilis* may lead to the discovery of novel mechanisms that regulate gene expression not only in endospore-forming pathogens but also in a wide variety of microorganisms.

**Grant:** 2R01GM055255-05A1  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** LEIGH, JOHN A PHD  
**Title:** Transcriptional Nitrogen Regulation in Methanococcus  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 1998/05/01-2007/12/31

DESCRIPTION (provided by applicant): Methanococcus maripaludis is an ideal model organism for the study of transcriptional regulation in the Archaea. Through the study of a nitrogen assimilation regulon, novel aspects of transcriptional regulation are coming to light. A complete system has been identified for detailed study: a novel repressor protein NrpR, an inducer (2-oxoglutarate) that modulates NrpR activity, three regulated promoter regions, and three different nitrogen sources that result in different regulatory states. Each promoter region contains a different configuration of operator sites and has a different regulatory outcome. A robust set of genetic tools allows for testing the effects of various manipulations on regulatory outcome in vivo. The system provides the opportunity to understand in detail a particular system of transcriptional regulation in the Archaea. Experiments will determine the subunit structure of the NrpR-DNA complexes, the binding properties of NrpR to each promoter region, how 2-oxoglutarate modulates binding, and how regulatory outcome is determined. In addition genetic studies will be carried out to identify the domain of NrpR that binds 2-oxoglutarate and the domain that mediates multimer formation. Knowledge of regulation in the Archaea will be greatly increased. Comparison with known mechanisms in the Bacteria and Eukarya may reveal fundamental similarities or differences in regulatory mechanisms. Archaeal transcription is a good model for eukaryotic transcription due to its distinct relatedness but relative simplicity. These studies in Archaea have wide importance, as regulatory mechanisms are central to human development and disease.

**Grant:** 2R01GM056695-06A1  
**Program Director:** MARINO, PAMELA  
**Principal Investigator:** POPHAM, DAVID L PHD  
**Title:** PEPTIDOGLYCAN SYNTHESIS IN BACILLUS SUBTILIS  
**Institution:** VIRGINIA POLYTECHNIC INST AND ST UNIV BLACKSBURG, VA  
**Project Period:** 1998/01/01-2008/04/30

DESCRIPTION (provided by applicant): Synthesis of the peptidoglycan cell wall is an essential process in most bacteria and is an effective target for antibiotics. The activities of the major enzymes, the penicillin-binding proteins (PBPs), involved in peptidoglycan polymerization are known, but the specific mechanisms by which the multiple proteins in this family control cell shape and division are not clear. The long-term objectives of the proposed studies are characterization of the functional domains of the major PBPs of *Bacillus subtilis* and identification of interactions between these and other proteins. Studies of peptidoglycan synthesis during both vegetative growth and spore formation will be undertaken. An understanding of the network of proteins involved in polymerizing particular cell wall structures will reveal new potential targets for antibiotic development. Knowledge of the processes of spore peptidoglycan formation and of spore peptidoglycan degradation during germination will contribute to the development of methods for spore killing. Such methods will be generally applicable to defense against spore-based biological weapons. Variant class A PBPs will be produced, including active site mutants, truncated proteins, and chimeric proteins containing domains from related proteins, and their abilities to carry out the various functions of the major *B. subtilis* class A PBP, PBP1, will be assessed. Enzymatic activities and effects on phenotypic properties (cell length, cell diameter, growth rate, peptidoglycan structure, and localization of PBP1) will be measured. Similar studies will examine the role of PBP2c in spore wall synthesis. Immunoprecipitation, assays for PBP and autolysin activities, defined mutant strains, western blotting, and mass spectrometry will be used to identify proteins that interact with class A PBPs during cell growth, division, and sporulation. Correlations will be made between disruptions of protein-protein interactions and changes in phenotypic properties. Cellular localization and protein-protein interactions of class B PBPs required for cell shape determination, cell division, and spore formation will be determined using immunofluorescence microscopy and immunoprecipitation.



<b>Grant:</b>	2R01GM058439-05	
<b>Program Director:</b>	LOGRASSO, PHILIP	
<b>Principal Investigator:</b>	WONG, CHI-HUEY	PHD CHEMISTRY, OTHER
<b>Title:</b>	RNA as a Target for Intervention	
<b>Institution:</b>	SCRIPPS RESEARCH INSTITUTE	LA JOLLA, CA
<b>Project Period:</b>	1999/02/01-2007/11/30	

A major focus of the research activities is to use the programmable one-pot synthesis methodology to create aminoglycoside libraries for screening and to modify the sugar domain of natural products know to target RNA in order to improve or alter their specificity.

**Grant:** 2R01GM058560-03A1  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** RUDD, KENNETH E AB  
**Title:** Analysis and annotation of the E. coli genome sequence  
**Institution:** UNIVERSITY OF MIAMI-MEDICAL CORAL GABLES, FL  
**Project Period:** 2000/02/01-2008/01/31

DESCRIPTION (provided by applicant): Genome sequencing projects have provided a foundation for a new biology centered around the molecular representation of genes and proteins as sequences and structures in computers. The parallel development of genome science, bioinformatics, the Internet and desktop "supercomputers" has helped bring this revolution to academic, industry and government labs worldwide. Unfortunately, the adverse impact of database errors on experimental science is easily demonstrated. The broad, long-term objective of this proposal is to continue to improve the reliability of the genome and proteome sequences of the model organism *Escherichia coli* K-12, leading to a Gold Standard Reference Strain for prokaryotic organisms, especially Gram-negative pathogens. This proposal focuses on improving the accuracy of the E. coli genome and proteome. The specific aims are: (1) to ensure the continued maintenance, improvement and expansion of EcoGene, a primary data repository for the continually revised E. coli genome and proteome sequences, and their annotations; EcoGene also serves as the systematic ORF nomenclature registry for E. coli K-12. EcoGene is part of an annotation-sharing collaboration among the Coli Genetic Stock Center at Yale, the Colibri database at the Pasteur Institute, and SWISS-PROT; (2) to establish two Indexer positions for expert electronic and legacy journal surveillance, to ensure that newly published and pre-released functional data about E. coli genes is entered promptly and accurately into EcoGene, then released to the public, and partner databases; (3) to augment electronic data collection with bioinformatics analysis to (a) discover new evolutionary relationships, thus improving functional predictions and (b) detect-and-report internal and external database errors, including DNA and protein sequence errors, often detected and resolved during analysis-anomaly-refinement-reanalysis (AARR) cycles; and (4) to use laboratory studies to (a) resolve remaining DNA frameshift errors in the E. coli K-12 genome by re-sequencing, (b) verify ambiguous protein starts, and (c) verify the secreted (periplasmic and outer membrane) proteome. The accurate annotation of the E. coli genome is necessary in its own right as the most well-understood cellular organism, and to provide the foundation for the analysis of bacterial genomes whose characterizations will be crucial for the development of biological and chemical defense against bacterial bioterrorism.

**Grant:** 2R01GM058750-06  
**Program Director:** TOMPKINS, LAURIE  
**Principal Investigator:** NUDLER, EVGENY A MS  
**Title:** Transcription Termination and Its Control in Bacteria  
**Institution:** NEW YORK UNIVERSITY SCHOOL OF NEW YORK, NY  
MEDICINE  
**Project Period:** 1999/01/01-2007/12/31

**DESCRIPTION** (provided by applicant): Transcription termination is a process whereby the elongation complex dissociates into RNA transcript, DNA template, and RNA polymerase (RNAP) in response to intrinsic DNA signals or specific factors. Both, intrinsic termination and factor-dependent termination are an integral part of the transcription cycle and play crucial role in regulating gene expression of bacteria and phages. Classical examples of such regulation include attenuation of transcription of biosynthetic operons and phage gamma-antitermination. The long-term objective of the proposed work is to provide a comprehensive structural and mechanistic description of intrinsic and Rho-dependent transcription termination processes in *Escherichia coli* and the mechanism of their positive and negative regulation by host factor NusA and phage XN protein, respectively. It is further proposed to examine the attenuation mechanism that controls nucleotide biosynthesis in *Bacillus subtilis*. Specific aims are: Complete studies on the mechanism of intrinsic termination and its control by N. The general mechanism of intrinsic termination and its regulation by N and NusA is now understood. However important details of the process remain unresolved. These include the positioning of N, NusA, and other Nus factors in the elongation complex, conformational changes in RNAP that accompany the termination process, and the active role of certain RNAP domains in termination. Experiments are proposed to address those questions. Analyze the mechanism of Rho-dependent transcription termination and its control by N. The rearrangement of protein-RNA and protein-DNA contacts in the elongation complex during termination by Rho will be monitored. Experiments are proposed to determine the effect of gamma N antitermination factor on the protein-nucleic acids interactions in the Rho-termination complex. Analyze the termination control mechanism of purine operons in *Bacilli*. Experiments are proposed to examine the role of newly discovered "riboswitches", i.e. natural regulatory RNA aptamers that directly sense small molecules, in regulation of purine operons in *B.subtilis*.

**Grant:** 2R01GM058794-05A2  
**Program Director:** ZATZ, MARION M.  
**Principal Investigator:** NEWTON, AUSTIN PHD  
**Title:** Sensor kinase regulation in Caulobacter differentiation  
**Institution:** PRINCETON UNIVERSITY PRINCETON, NJ  
**Project Period:** 1999/01/01-2008/05/31

DESCRIPTION (provided by applicant): Asymmetric cell division is a fundamental mechanism for differentiation and the generation of new cell types. The gram negative, alpha-proteobacterium *Caulobacter crescentus* follows an invariant pattern of unequal cell division to produce two progeny cells with different structures and developmental fates. This organism is now recognized as an ideal model system for the study of how cellular asymmetry is established and its role in developmental regulation. An understanding of these processes is the long-term goal of the research proposed in this application. Genetic analysis has shown that developmental events in these cells are tightly coordinated with cell cycle progression by networks of essential signal transduction pathways mediated by the two-component family of proteins. Remarkably, the sensor kinases that initiate these pathways are, like the morphogenic events they control, spatially localized at the cell poles. A central focus of the proposed work is to understand the molecular mechanisms responsible for the subcellular localization of the PleC, DivJ, DivL and CckA kinases and the role of localization in kinase function and regulation. The aims of the proposed experiments are to: (1) carry out a detailed genetic and biochemical analysis of the PleC kinase and its cognate essential response regulator DivK to define their roles in the regulation of polar morphogenesis and cell division; (2) identify the sequences within PleC and other cell cycle-regulated kinases that specify their subcellular localization and examine the role of localization in developmental regulation; and (3) identify elements of the cellular machinery responsible for subcellular localization of these signal transduction proteins.

**Grant:** 2R01GM058822-05A1  
**Program Director:** JONES, WARREN  
**Principal Investigator:** VAN DER DONK, WILLEM A PHD  
**Title:** Biosynthetic Enzymes Involved in Lanthionine Formation  
**Institution:** UNIVERSITY OF ILLINOIS URBANA- CHAMPAIGN, IL  
CHAMPAIGN  
**Project Period:** 1999/02/01-2008/07/31

DESCRIPTION (provided by applicant): Lantibiotics are a group of ribosomally synthesized peptide antibiotics that are post-translationally modified to their bioactive forms. These modifications include initial dehydration of serine and threonine residues followed by cyclization of cysteines onto the dehydro amino acids generated in the first step. Previous in vivo studies revealed that the enzymes responsible for these processes lack absolute substrate specificity. Thus, protein engineering using chemically synthesized unnatural peptide substrates may provide a valuable tool to study structure-function relationships in lantibiotic biosynthesis, and to establish their molecular mechanism(s) of cytotoxicity. In order to achieve these goals, the activities of the enzymes involved in lactacin 481 biosynthesis will be investigated in the first two specific aims. These studies will focus on the interaction of the enzyme with their substrates and the mechanism of dehydration and cyclization. Genetic protein engineering is limited to the 21 physiological amino acids. However, since the size of the prepeptides of lantibiotics is well within the limit of solid phase peptide synthesis, the pool of available amino acids that can be used for "chemical protein engineering" is increased dramatically. Thus, in the fourth specific aim, the natural peptide substrates for post-translational modification will be altered at specific positions by substitution with synthetic unnatural amino acid analogs. This approach may be very powerful to gain insight into the mechanism of biosynthesis of the lantibiotics. Moreover, these studies may produce novel variants of the natural lantibiotics with potentially interesting biological activities.

**Grant:** 2R01GM059281-07  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** YAFFE, MICHAEL B PHD  
**Title:** Neutrophil Priming in Trauma and Sepsis  
**Institution:** MASSACHUSETTS INSTITUTE OF CAMBRIDGE, MA  
TECHNOLOGY  
**Project Period:** 1999/05/01-2008/04/30

DESCRIPTION (provided by applicant): Neutrophil priming and activation following trauma and sepsis is a key event implicated in causing Adult Respiratory Distress Syndrome (ARDS), Multi-Organ Failure Syndrome (MOSF), and ischemia-reperfusion injury. Priming of the respiratory burst by cytokines following injury and sepsis results in excessive superoxide production by the NADPH oxidase leading to auto-inflammatory tissue damage. Many of the molecular mechanisms involved in priming and activation of the NADPH oxidase, however, remain poorly defined. Our long-term goal is to develop a detailed molecular understanding of how protein kinase and lipid kinase signaling pathways, including the PI 3-kinase pathway, the p38MAPK pathway and the Erk1/2 pathways regulate the assembly, subcellular targeting, and activity of the neutrophil NADPH oxidase during priming and activation. Our previous work and preliminary observations identified PX domains in the p47phox and p40phox subunits as modular protein domains that bind to specific lipid products of PI 3-kinase, and showed that the priming agents PAF and TNF $\alpha$  induced the assembly of a p47phox:p67phox:p40phox heterotrimeric complex in the cytoplasm of primed but un-activated cells. In the studies outlined in this proposal we will investigate what roles the PX domains play in neutrophil priming and activation, and determine the protein kinase signaling pathways and molecular mechanisms involved in priming-induced heterotrimer formation. The results from these studies may assist in the development of novel diagnostic or therapeutic reagents aimed at limiting the auto-inflammatory tissue damage patients suffer as a result of sepsis and trauma.

**Grant:** 2R01GM059295-06  
**Program Director:** TOMPKINS, LAURIE  
**Principal Investigator:** SEVERINOV, KONSTANTIN V PHD  
**Title:** Phage-induced modifications of RNA polymerase  
**Institution:** RUTGERS THE ST UNIV OF NJ NEW BRUNSWICK, NJ  
BRUNSWICK  
**Project Period:** 1999/05/01-2008/04/30

**DESCRIPTION** (provided by applicant): Our long-term goal is to understand the function and regulation of cellular RNA polymerase (RNAP) in molecular detail. Bacteriophages evolved elaborate mechanisms to regulate transcription of bacterial host to serve viral needs. The number of phage-encoded transcription regulators exceeds the number of bacterial regulators by orders of magnitude. Phage regulatory systems are usually compact, robust and efficient. Studies of a handful of phage-induced modifications of host RNAP provided important paradigms of regulation of gene expression that are applicable to bacteria and higher organisms. The goal of this research is to study phage-induced modifications of bacterial host RNAP and the role of these modifications in viral development. In vitro, RNAP sites that are targeted by phage regulators will be identified and the mechanisms of action of phage regulators will be determined. In vivo, genetic and genomic approaches will be used to understand the biological consequences of RNAP modifications by phage-encoded inhibitors. The following model systems will be analyzed: The T4 phage. Molecular mechanism of termination factor Ale will be studied. The mechanism of negative regulation of host and early viral genes and positive regulation of middle viral genes by T4 AsiA will be determined. The role of ADP-ribosylation of RNAP alpha in regulation of early and middle viral transcription will be studied. The T7 phage. The role of RNAP beta phosphorylation by T7 gp0.7, the molecular mechanism of E. coli RNAP transcription inhibition by T7 gp2, and the role of gp2 in viral DNA packaging will be investigated. The Sp6 phage. Sp6-encoded inhibitor(s) of S. typhimurium RNAP will be purified and characterized. The Xp10 phage. Molecular mechanism of a novel Xp 10-encoded antitermination factor p7 will be identified. Global transcription profiling will be used to better understand gene expression strategy of Xp10, a highly unusual phage that appears to combine the regulatory paradigms of well-studied T7 and lambda phages. The thematic unity of this application stems from its focus on negative regulation of bacterial RNAP by covalent modifications or RNAP-interacting proteins during viral development. Studies of phage-encoded proteins and modifications of host RNAP will lead to deeper understanding of viral biology. On the other hand, phage-encoded transcription inhibitors will be used as molecular probes to better understand RNAP mechanism and regulation and to uncover RNAP sites that can be targets for drug design.

**Grant:** 2R01GM059776-06  
**Program Director:** SHAPIRO, BERT I.  
**Principal Investigator:** GOODRICH-BLAIR, HEIDI PHD  
**Title:** MOLECULAR MECHANISMS OF X. NEMATOPHILA-NEMATODE INTERACT  
**Institution:** UNIVERSITY OF WISCONSIN MADISON MADISON, WI  
**Project Period:** 1999/05/01-2008/04/30

DESCRIPTION (provided by the applicant): The long-term goal of our research is to elucidate molecular mechanisms mediating host-microbe interactions and to learn how microbial processes affect the outcome of these relationships. The model bacterium we use for these studies is *Xenorhabdus nematophila*, which is both a mutualist (of nematodes) and a pathogen (of insects), and therefore an excellent model to understand both types of relationships. This proposal focuses on the mutualism between *X. nematophila* and the nematode and complements separately funded work in our lab on *X. nematophila* pathogenesis. Examining the mutualistic interactions of *X. nematophila* and its nematode host will expand our understanding of how animals evolve and maintain specific and dependent relationships with their micro-flora. The specific aims of this proposal are geared toward genetically and biochemically characterizing several of the colonization initiation factors we have identified, as well as their interactions with each other. Specific Aim 1 will test our hypotheses that membrane-localized colonization factors function in adherence to nematode cells, metal transport, and/or signal transduction. The results of these studies will elucidate the roles of Nil (nematode intestine localization) proteins in colonization events, shed light on the possible functions of Nil homologs of gram-negative pathogens in initiating infections, and further our understanding of molecular mechanisms mediating host-range specificity. Specific Aim 2 is designed to dissect the functions of two regulators required for colonization, a sigma factor and a regulatory RNA. These experiments will yield insight into the signals perceived by *X. nematophila* during colonization, further define a colonization-specific regulon, and expand our knowledge of the role of a ubiquitous transcription factor in the natural ecology of a bacterium.



**Grant:** 2R01GM059969-06  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** ROMEO, TONY  
**Title:** Mechanism of CsrA-mediated global regulation  
**Institution:** EMORY UNIVERSITY ATLANTA, GA  
**Project Period:** 1999/08/01-2007/11/30

Insight into post-transcriptional regulatory mechanisms of bacterial gene expression will be sought through the study of a novel paradigm in global regulation, the carbon storage regulatory system (Csr) of *Escherichia coli*. Csr includes an RNA-binding protein, CsrA, that regulates translation and/or modulates the stability of specific mRNAs, and two small non-coding RNA molecules, CsrB and CsrC, which antagonize CsrA activity. It is hypothesized that CsrB and CsrC interfere with CsrA-mediated regulation by competing with regulated mRNAs for CsrA binding. In *E. coli*, CsrA affects metabolism, physiology and multicellular behavior on a broad scale, repressing certain genes expressed during the transition from the exponential to stationary phase of growth and activating various genes expressed during exponential phase. CsrA homologues are widely-distributed among eubacteria and regulate the expression of virulence factors in both plant and animal pathogens. Thus, the proposed studies will also provide fundamental understanding of the regulation of bacterial physiology and pathogenesis, and may suggest novel therapeutic approaches for bacterial infections. Specific aims of this proposal are: 1) To further elucidate the molecular mechanisms responsible for CsrA-mediated activation or inhibition of gene expression. This will include determination of the sequence and structural requirements for mRNA recognition and the mechanism by which CsrA-mediated translational inhibition leads to degradation of target transcripts. 2) We will assess the molecular mechanisms and regulatory roles of small noncoding RNAs in CsrA antagonism. The isolated CsrA-RNP complex will be characterized to determine the RNA segments involved in CsrA binding. A novel RNA that complexes with CsrA will be characterized. 3) We will delineate the genetic, physiological and environmental factors to which the Csr system responds. We will examine the physiological and molecular basis by which flux through the cysteine biosynthesis pathway regulates *csrA* gene expression. The long-range basic objectives of these studies are to fully understand the regulatory components and genetic circuitry, molecular mechanisms, and biological functions of the Csr system.

**Grant:** 2R01GM060268-05  
**Program Director:** LEWIS, CATHERINE D.  
**Principal Investigator:** UHLENBECK, OLKE C PHD  
BIOCHEMISTRY:PHYSICAL  
**Title:** Biophysical Chemistry of a DEAD/H Protein  
**Institution:** NORTHWESTERN UNIVERSITY EVANSTON, IL  
**Project Period:** 2000/06/01-2008/07/31

DESCRIPTION (provided by applicant): The long term goal of this project is to obtain a mechanistic understanding of a large, ubiquitous class of proteins, termed DEXD/H proteins, which participate as essential factors in many cellular processes involving RNA. DEXD/H proteins are believed to act as RNA helicases to catalyze conformational changes in large RNAs, however, other functions have been proposed. The intent is to perform biochemical and biophysical experiments on purified proteins that will complement extensive efforts by many other groups applying molecular genetic and molecular biological methods to the same proteins in their more complex physiological setting. This project initially chose E. coli DbpA as a model for detailed study, because of its exceptional experimental tractability. Not only is it biochemically well-behaved, but, in contrast with all other DEXD/H proteins, it shows very tight binding and high specificity for its target RNA, which simplifies structural and biochemical experiments. We have established that DbpA interacts with RNA in a unique manner, shown that it has helicase activity and understood how the high affinity and specificity is achieved. Current aims include (1) mechanistic experiments to understand how DbpA acts as a helicase and whether it is designed to only open a few base pairs. (2) biochemical experiments defining how DbpA interacts with 23S rRNA and (3) molecular microbiological experiments designed to determine the step in the bacterial ribosome assembly pathway where DbpA acts. Finally, high throughput RNA binding, ATPase and RNA helicase assays in microtiter plates will be developed, in order to assay many other DEXD/H proteins, including the 18 family members involved in yeast ribosome assembly.

**Grant:** 2R01GM060329-04A1  
**Program Director:** PREUSCH, PETER C.  
**Principal Investigator:** ADAMS, MICHAEL W.W. PHD BIOCHEMISTRY  
**Title:** Novel Responses to Oxygen by Anaerobic Microorganisms  
**Institution:** UNIVERSITY OF GEORGIA ATHENS, GA  
**Project Period:** 2000/03/01-2008/02/28

DESCRIPTION (provided by applicant): By definition, aerobic organisms, both prokaryotic and eukaryotic, require molecular oxygen for energy conservation. This is a mixed blessing, however, as extremely reactive oxygen derivatives are produced during normal metabolism that can damage all cellular components. These so-called reactive oxygen species (ROS) have been implicated in a wide variety of chronic and infectious human diseases, including cancer, Alzheimer's disease, arthritis and AIDS, yet ROS are also used as a defense system against pathogens and in signal transduction pathways. Understanding the responses of microbes to oxygen has direct ramifications for the treatment of diseases caused by anaerobic pathogens. In 1999 we proposed that anaerobes have a novel response to ROS in which a non-heme iron protein termed superoxide reductase (SOR) played a key role. SOR was characterized from the hyperthermophilic anaerobe, *Pyrococcus furiosus*, and over the prior funding period it has been established using structural and spectroscopic approaches that SOR is uniquely suited to catalyze superoxide reduction. Using DNA microarrays to all 2065 ORFs in the complete *P. furiosus* genome, it was shown that the genes encoding SOR and related proteins are all expressed at significant levels in the absence of any oxidative shock. *P. furiosus* is therefore continuously 'armed' and ready to deal with ROS exposure. This is a first line of defense, however, as DNA microarray analyses show that the complete response to oxidative stress requires the induction of a large number of novel proteins (encoded by conserved/hypothetical genes), some of which are also induced by growth at sub-optimal temperatures. In the proposed research, the novel stress-regulated proteins, together with SOR and related reductases and oxidases, will be characterized with respect to their regulation, multiprotein complex formation, and catalytic functions using immunological, biochemical and structural analyses. A variety of complementary spectroscopic techniques, including EPR, ENDOR, MCD, resonance Raman, FTIR and X-ray absorption, will be utilized to probe the catalytic function of specific members of the stress-related pathways, with particular emphasis on SOR. The results will provide completely new insights into the stress responses of anaerobes, and provide strategies for determining the function of uncharacterized hypothetical algenes that typically account for half of a microbial genome.

**Grant:** 2R01GM060731-05  
**Program Director:** ECKSTRAND, IRENE A.  
**Principal Investigator:** DYKHUIZEN, DANIEL E PHD  
**Title:** An Evolutionary Analysis of Fimbriae in Enterobacteria  
**Institution:** STATE UNIVERSITY NEW YORK STONY BROOK STONY BROOK, NY  
**Project Period:** 2000/02/01-2008/02/29

DESCRIPTION (provided by applicant): The major emphasis of this proposal, which continues the work of the previous proposal, is to devise ways to determine which mutations are involved in adaptive evolution of bacteria as human pathogens. The model system used here is the adaptation of adhesins and other genes of *Escherichia coli* to extra intestinal infections, particularly urinary tract infections (UTI). The within-clonal genetic diversity of the major uropathogenic serotypes will be used to determine gene loci targeted by pathoadaptive mutations, i.e. gene changes that are selected in the environment when the organism is a pathogen. We will study in detail strains belonging to O18:K1:H7 serotype - a major uropathogenic clone. Nucleotide polymorphisms within the gene clusters encoding various adhesive fimbria will be determined and used to characterize ancestral/descendent relationships within the clone. Then, two ancestral and two descendant strains will be surveyed for additional mutational changes in up to one megabase of genome by using the newly developed technique, GIRAFF. In GIRAFF, sized fragments from two strains are melted, rehybridized and treated with the mismatch-specific endonuclease, CEL I, that is capable of cutting mismatched DNA regions with high specificity and sensitivity. The CEL I-specific bands are then identified by Southern blot hybridization using multi-kb DNA probes. Within the 20% of the *E. coli* genome to be surveyed, about two-dozen synonymous mutations are expected and will be used to date the clone. All other mutations will be analyzed as potential pathoadaptive changes. The genes in which the nonsynonymous mutations are found and the intervening regions where mutations are found will be tested to see if similar mutations are found in the same regions of DNA in six other uropathogenic clones. Those regions commonly found with mutations within each of these other clones will be assumed to important in pathogenesis. The functional effects of these mutations will be investigated. A subset of these pathoadaptive loci will be sequenced in our collection of 125 *E. coli* strains, which includes both commensal and pathogenic strains, to see if our new analytic technique, zonal analysis, will confirm that these changes are pathoadaptive. If so, this approach can be used to discover pathoadaptive loci from the many expected to be sequenced genomes of *E. coli*.

**Grant:** 2R01GM061019-05  
**Program Director:** MARINO, PAMELA  
**Principal Investigator:** YOUNG, KEVIN D  
**Title:** Bacterial cell wall synthesis, shape and septation  
**Institution:** UNIVERSITY OF NORTH DAKOTA GRAND FORKS, ND  
**Project Period:** 2000/03/01-2008/06/30

**DESCRIPTION** (provided by applicant): In addition to protecting bacteria from lysis, the peptidoglycan cell wall plays an important, underappreciated role in several physiological processes. These range from the basic biological functions of creating cellular polarity, influencing differentiation and impeding virus entry, to contributing to host attachment, toxin production and recognition by the innate immune response. Even less well understood are the contributions of bacterial morphology to nutrient accumulation, attachment, motility, chromosome segregation, predation, biofilm formation and virulence. Our long-term goal is to understand the structure, synthesis, regulation and functional implications of peptidoglycan and the enzymes that create and modify it. In particular, we've been searching for the physiological functions of the low molecular weight penicillin binding proteins, a large family of peptidoglycan-modifying enzymes that are present in multiple forms and highly conserved across the bacterial kingdom. These enzymes interact with the bacterial division protein, FtsZ, to create cells of defined and uniform shape via reactions that are either independent of or which precede the now-classic division pathway initiated by FtsZ. Of great import is that newly described phenotypes in multiply-mutated strains allow questions of cell shape and polarity to be approached by genetic techniques not previously available for such studies. We propose to refine and characterize the functioning of this "morphological pathway" by pursuing the following specific aims: 1) characterize the role of FtsZ in generating and localizing inert peptidoglycan; 2) characterize the roles of peptidoglycan hydrolases in cell shape and integrity; 3) define how interacting helical structures propel growth of the wall and envelope; and 4) isolate and characterize suppressor mutants to define the morphological pathway in more detail. These goals will be realized by creating strains deficient in portions of the proposed pathway, by assaying current FtsZ mutants and creating new ones, and by isolating suppressor mutants to define additional components of the pathway.

**Grant:** 2R01GM061162-05A1  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** LEWIS, KIM A PHD  
**Title:** A GENOMICS APPROACH TO BIOFILMS  
**Institution:** NORTHEASTERN UNIVERSITY BOSTON, MA  
**Project Period:** 2000/09/01-2008/02/28

DESCRIPTION (provided by applicant): Our long-term goal is to elucidate the mechanism of biofilm tolerance to antibiotics. Our preliminary studies suggest that persister cells may be largely responsible for resistance of biofilms and stationary planktonic populations to killing by cidal antimicrobials. The main goal of this proposal is to identify genes responsible for the persister phenotype. This will enable us to directly test the persister hypothesis of biofilm resistance which promises to solve this long-standing riddle, and will provide a new paradigm for the understanding and treatment of biofilm infections. We will use a number of complementary approaches to identify persister genes. We were able to isolate persisters from a high-persistence (hip) strain of *E. coli* by lysing the bulk of cells with ampicillin, and obtained a preliminary gene expression profile. A detailed time-dependent gene profile of ampicillin treatment will be obtained, providing data for a comprehensive cluster analysis that will indicate candidate persister genes. We will isolate naive persisters using cell sorting with GFP linked to genes that are likely to be expressed in these cells. Additionally, using DNA arrays, we will identify an overlapping set of genes differentially expressed in cells treated with unrelated antibiotics. Persister genes are expected to be among those affecting death and survival. In an independent approach, persister genes will be identified by selection for increased tolerance from a recombinant genomic library. Candidate genes from these approaches will be tested in uniformly constructed strains, each carrying a deletion; and overexpressing the gene from a controllable promoter. Tests with a set of antibiotics will indicate genes that affect persister production in planktonic cultures. Biofilms will then be prepared from persister-deficient or overproducing strains, and tested for tolerance with cidal antibiotics. Correlation between persister status of a strain and biofilm tolerance will provide a definitive test for the persister hypothesis. Identified persister genes will then enable a study of their mechanism of action, which will begin with obtaining an expression profile from strains deficient in; and overproducing the protein of interest. These studies will form the basis for understanding biofilm infections and developing drugs that target persister proteins.

**Grant:** 2R01GM061258-05  
**Program Director:** LEWIS, CATHERINE D.  
**Principal Investigator:** MOORE, PETER B  
**Title:** Structure and Physical Properties of RNA  
**Institution:** YALE UNIVERSITY NEW HAVEN, CT  
**Project Period:** 2000/07/01-2008/06/30

**DESCRIPTION** (provided by applicant): The principle activity for which this application seeks support is determination of the structures of physiologically important RNAs by NMR and X-ray crystallography. The ultimate objective is a chemical understanding of the biological properties of the RNAs studied. Since RNAs play critical roles in gene expression, the knowledge sought is fundamental to our understanding of processes vital to all organisms, including humans. Two specific problems are to be investigated: the regulation of ribosomal protein synthesis in bacteria, and the pseudouridylation of ribosomal RNA transcripts in eukaryotes. In bacteria, ribosomal protein synthesis is feed-back regulated at the translational level by mechanisms that depend on interactions between specific ribosomal proteins and sequences within the mRNAs that encode them, many of which are polycistronic. The two such systems of immediate concern are the *spc* operon/S8 system, and L10 operon./L10 system, but time permitting, others will be investigated; e.g. the *alpha* operon/L4 system. The mRNA sequence critical for the regulation of each ribosomal protein operon will be determined as precisely as possible. Structures will be obtained of the complexes that form between these RNA sequences and the ribosomal proteins that bind to them. Hypotheses about the mechanism of translational repression, formulated on the basis of those structures, will then be tested biochemically. Since bacteria regulate ribosomal protein synthesis by mechanisms unlike those used by eukaryotes, it is conceivable that new ways of specifically inhibiting bacterial growth will be revealed. The second problem to be investigated is posed by the *boxH/ACA* snoRNP system responsible for pseudouridylating rRNA transcripts in higher organisms. The RNA components of these snRNPs are postulated to interact with sequences in rRNA transcripts in a way that has no precedent in the literature. Biochemical experiments will be done to determine whether RNA/RNA interactions of the sort hypothesized are possible, and structures will be obtained for a snoRNA, with and without an appropriate rRNA sequence bound.

**Grant:** 1R01GM065248-01A2  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** BOONS, GEERT-JAN PHD  
**Title:** Cellular Activation Induced by Multivalent Ligands  
**Institution:** UNIVERSITY OF GEORGIA ATHENS, GA  
**Project Period:** 2004/01/01-2007/12/31

**DESCRIPTION** (provided by applicant): This project is a strategic component of our long-range research goals, which are to identify molecular mechanisms responsible for the contribution of toxic bacterial cell wall components to septicemia, and to use this knowledge to develop efficacious treatments. The project has four specific aims designed to test our central hypothesis that PGN induces hetero-dimerization of Toll like receptor (TLR) 2 and TLR6, leading to cellular activation. The proposed role of the cluster differentiation antigen CD 14 is to enhance cellular responsiveness to PGN by immobilizing it on the cell surface through interactions with multiple CD14 receptors. Furthermore, the available data suggest that CD14 recognizes the carbohydrate moiety of PGN, whereas TLR2 and TLR6 recognize the peptide portion. To test these hypotheses, a series of partial structures of PGN will be synthesized, which will be attached to synthetic polymers and well-defined multivalent scaffold to give a range of poly- and multivalent ligands. These ligands will be tested for agonist activity in human monocytic cells using as primary read-outs production of TNFalpha protein, activation of NF-kappaB and MAP kinases, and expression of TNFalpha mRNA. To determine the ligand specificities of the receptors, parallel studies will be performed with human cells transfected with CD14, TLR2, TLR6, and combinations of the three receptors. Ligands that bind to the cells yet lack agonist activity will be evaluated for their ability to antagonize the proinflammatory effects of PGN. The binding affinities of the synthetic ligands will be determined by measuring their ability to prevent binding of radiolabeled reference compounds in a cell-based assay. Correlations between structures of individual multivalent ligands, their relative binding affinities for CD14, TLR2, and TLR6 and agonist or (partial) antagonist properties will permit identification of the ligand requirements (part structure, valency and linker) of the individual receptors, demonstrate the importance of multi-valency for high avidity binding, and establish how the different receptors cooperate to induce cell signaling. Finally, confocal microscopy and fluorescence resonance energy transfer (FRET) studies will be employed to determine whether PGN and the multivalent ligands induce clustering of the relevant receptors. The results of the proposed studies will provide a scientific foundation for the development of therapeutic strategies to increase the survival rates of patients with Gram-positive septicemia.



**Grant:** 1R01GM065503-01A2  
**Program Director:** SCHWAB, JOHN M.  
**Principal Investigator:** LOVELY, CARL J PHD  
**Title:** Approaches to Bioactive Aminoimidazole Natural Products  
**Institution:** UNIVERSITY OF TEXAS ARLINGTON ARLINGTON, TX  
**Project Period:** 2004/01/01-2008/12/31

DESCRIPTION (provided by applicant): The long-term goals of the research described in this submission are to provide a set of synthetic transformations for elaborating simple imidazoles into biologically relevant natural products and congeners. Two distinct projects are proposed that rely on mechanistically diverse reactions, but all involve, in one way or another, the disruption of the aromaticity of the imidazole ring. The first project seeks to develop the inter- and intramolecular Dieis-Alder reaction of 4-vinylimidazoles as a means to construct polycyclic molecules that may be useful en route to natural products, in particular the oroidin derived pyrroloimidazole class of compounds isolated from marine sponges. This reaction is expected to feature as the key step in the assembly of a number of targets including the Agelas alkaloids (e.g., ageliferin), which possess antibacterial properties, and the more complex palau'amine and related targets, konbu'acidin A, styloguanidine and axinellamine A. Palau'amine, a hexacyclic, bisguanidine-containing alkaloid, is a potent immunosuppressant, exhibiting a nanomolar IC50 in the mixed lymphocyte reaction. Furthermore, palau'amine showed significant activity against P-388 and A-549 cancer cell lines, with IC50's of 0.2 and 0.5 fM respectively. The related acylated congener, konbu'acidin A, inhibits cyclin dependent kinase 4 and thus may prove useful as an anti-cancer lead, whereas styloguanidine is a chitinase inhibitor. The second project will focus on the recently discovered dimethyldioxirane induced oxidative rearrangement of bicyclic imidazoles (tetrahydrobenzimidazoles) to spiro imidazolones. It is proposed to investigate the scope and limitations of this potentially important reaction. In particular, the influence of both imidazole substituents on nitrogen and at C2 will be investigated along with the role of peripheral substituents at C4 and C5 on the stereochemical outcome of the rearrangement. It is anticipated that this reaction will play a pivotal role in approaches to palau'amine, konbu'acidin A, styloguanidine and axinellamine A. Also, it is planned to establish the applicability of the rearrangement to imidazo[4,5-b]pyridines, if successful, the rearranged products might function as key intermediates in approaches to several monomeric oroidin-derived alkaloids, such as phakellin, saxitoxin and cantharelline. The final projects will apply the methodology developed during the exploratory phases of this program to a number of the dimeric oroidin-derived marine alkaloids (i) ageliferin, (ii) axinellamine A, (iii) palau'amine, (iv) konbu'acidin A and (v) styloguanidine.

**Grant:** 1R01GM066025-01A2  
**Program Director:** ECKSTRAND, IRENE A.  
**Principal Investigator:** DANGL, JEFFREY L. PHD GENETICS  
**Title:** Diversity and evolution of P syringae type III effectors  
**Institution:** UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC  
HILL  
**Project Period:** 2004/01/01-2007/12/31

DESCRIPTION (provided by applicant): Identification of virulence factors is a necessary first step to study the mechanisms and evolution of pathogenicity. We propose to compare the type III disease effector proteins from a phylogenetically diverse set of plant pathogenic Pseudomonads chosen to represent the most broad distribution of the species possible. *P. syringae* is an ideal model organism to study the distribution and evolution of type III effectors. Type III effectors interact with cellular host targets, and modulate host defense responses or metabolism in a manner conducive to pathogen proliferation. Most of the *P. syringae* effectors have not yet been characterized, and we remain naive as to the collective diversity of host cellular functions they manipulate. Although the functions of *P. syringae* effector proteins during disease remain poorly understood, they can often be monitored via their phenotypes following in planta expression of a given type III effector. Bacterial pathogens of both animals, such as *Salmonella* spp., *Yersinia* spp., *Shigella* spp. and pathogenic *E. coli* also rely on type III secretion systems for pathogenesis. Thus, our results will inform studies of these bacteria and their animal hosts, including humans. Because *P. syringae* is pathogenic on a variety of distantly related plant hosts, many of which can be genetically manipulated, our system has advantages over models of type III pathogenesis of animals, which generally focus only on strains pathogenic on phylogenetically related mammalian hosts. We have devised and implemented a high throughput Fluorescence Activated Cell Sorter (FACS) based experimental approach to capture all of the type III effectors from the genome of any given *P. syringae* isolate. We chose 13 *P. syringae* isolates that are pathogens of an evolutionarily diverse set of host plants. We intend to sieve through these 13 genomes to describe their suites of type III effectors. We further intend to begin dissection of their effects on host cell biology, using the easily manipulated *Arabidopsis* plant as a model where appropriate. This proposal is highly interdisciplinary, drawing on methodologies and expertise in microbiology, bioinformatics and plant-pathogen interactions in conjunction with high throughput bacterial cell sorting using the FACS. Our work will broadly impact the understanding of a widely distributed pathogenicity mechanism that affects both human health and agriculture. Additionally, our work may have relevance in biodefense with respect to basic understanding of pathogenesis in bacteria that can potentially be weaponized.

**Grant:** 1R01GM066130-01A1  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** WOLFE, ALAN J  
**Title:** Acetyl Phosphate as a Global Stress Signal  
**Institution:** LOYOLA UNIVERSITY CHICAGO MAYWOOD, IL  
**Project Period:** 2004/05/01-2008/04/30

DESCRIPTION (provided by applicant): The network of two-component signaling pathways controls a multitude of genes in response to diverse environmental signals. For example, during their colonization of host tissue, bacterial pathogens use multiple two-component pathways to ensure they express the proper subset of virulence factors. Less well understood is the impact made on this signaling network by the small molecule acetyl phosphate. We recently reported that acetyl phosphate affects expression of about 100 genes involved in the synthesis of flagella, type 1 pili, capsule and stress effectors - structures implicated in biofilm development. In the 1st aim, we will combine microarray technology, bioinformatics and transcriptional analyses to obtain a comprehensive list of uropathogenic and non-pathogenic *E. coli* genes that respond to acetyl phosphate and the transcription factors that mediate that response. If we find that most of these transcription factors are response regulators, then we will have obtained evidence that acetyl phosphate can influence gene expression through response regulators. An increasing number of published reports rely either explicitly or implicitly on the "fact" that acetyl phosphate acts as a phospho-donor for response regulators in vivo. Although alternative explanations exist, in our opinion, the "direct phospho-donor" model best explains the existing data. Most, but not all, of the connections predicted by this hypothesis have been documented and no new players or mechanisms need to be found or envisioned. In the 2nd aim, we propose to make the final, critical connection - to determine whether in vivo that a specific response occurs because acetyl phosphate donates its phosphate to a specific response regulator. We also will use biochemical means to test the hypothesis that molecular crowding can increase the efficiency of the phosphorylation reaction, thereby increasing the capacity of acetyl phosphate to act as a phospho-donor in vivo. We possess evidence indicating that OmpR plays a previously unknown role required by cells that can synthesize acetyl phosphate. In the 3rd aim, we will elucidate the linkage between acetyl phosphate and OmpR. We will dissect an OmpR-dependent phenotype that requires the synthesis of acetyl phosphate - the propensity of ompR ackA mutants to lyse during late exponential phase. We will test the three specific hypotheses outlined in this aim, and we will identify the suppressor mutations that permit a small subset of cells to escape lysis. This approach should either verify that acetyl phosphate acts directly through response regulators or lead us to alternative explanations.

**Grant:** 1R01GM066934-01A2  
**Program Director:** LEWIS, CATHERINE D.  
**Principal Investigator:** WHITE, STEPHEN W  
**Title:** Recombination and fork progression in bacteriophage T4  
**Institution:** ST. JUDE CHILDREN'S RESEARCH HOSPITAL MEMPHIS, TN  
**Project Period:** 2004/08/01-2008/07/31

DESCRIPTION (provided by applicant): The repair of DNA lesions such as double strand breaks (DSBs) is crucial to the stability of the genome, and we are proposing to study two processes that are fundamental to the prevention and repair of DSBs in all cells. The first is recombination-dependent replication (RDR) that is now recognized as a central mechanism in DNA metabolism that operates in many DNA repair scenarios. The second process is replication fork progression and the rescue of stalled forks. Stalled forks can lead to DSBs, and they need to be rapidly dealt with in the cell. We are interested in the underlying mechanisms that operate in these two processes, and will therefore study them in a very simple, well characterized organism, namely bacteriophage T4. Phage T4 is an ideal system for the multidisciplinary approach that we have designed, and there are many similarities between T4 and eukaryotic proteins. Defects in repair mechanisms lead to the accumulation of mutations that eventually result in cancer, and the proposed studies in T4 are therefore directly relevant to human disease. Five T4 proteins will be studied, UvsX, UvsY, UvsW, Dda and MotA. The recombinatorial proteins UvsX and UvsY mediate the T4 homologous recombination reaction that is required for RDR. UvsW and Dda are helicases that translocate and/or unwind branched nucleic acid structures and have important roles in RDR and replication fork progression. Defects in helicases such as Bloom and Werner are known to cause cancer in humans, and there is evidence that UvsW and Dda may be functionally homologous to these molecules. Finally, structural studies suggest that the T4 transcription factor MotA binds DNA in a novel fashion that is shared by UvsW. The mechanisms of these five proteins will be studied at the molecular level by a coordinated approach involving X-ray crystallography and NMR spectroscopy to study their structures, in vitro methods to study their individual functions and interactions, and in vivo methods to understand their biological roles. A considerable body of preliminary data have been obtained for this project that includes two high resolution structures, crystals, purified proteins and functional information from T4 mutants. The P.I. will direct the structural studies, and the co-P.I. will direct the in vivo studies. The in vitro analyses will be performed in both laboratories as appropriate.

**Grant:** 1R01GM067672-01A1  
**Program Director:** WEHRLE, JANNA P.  
**Principal Investigator:** TSAI, FRANCIS T OTH  
**Title:** Structural Studies of Clp/Hsp 100 Molecular Chaperones  
**Institution:** BAYLOR COLLEGE OF MEDICINE HOUSTON, TX  
**Project Period:** 2004/01/01-2008/12/31

DESCRIPTION (provided by applicant): Proteins must fold correctly in order to attain biological function. Concurrently, protein aggregation and misfolding are key contributors to many devastating human diseases such as Alzheimer's disease, prion-mediated infections, type II diabetes, and cystic fibrosis. While "conventional" molecular chaperones assist protein folding by promoting the "forward" folding or preventing protein aggregation, they are unable to promote the disaggregation of already aggregated proteins such as amyloids, which are associated with certain human diseases. Bacterial ClpB and its eukaryotic homolog Hsp104 are essential proteins of the heat-shock response, form large ring-like structures, and belong to the Hsp100 family of ATPases associated with diverse cellular activities (AAA+). Unlike any other chaperone, including other members of the Hsp100 family, ClpB/Hsp104 has the remarkable ability to promote the disaggregation of already aggregated, stress-damaged proteins. The underlying mechanism is currently unknown due to the lack of high-resolution structural information. The long-term objective is to understand the molecular mechanism by which members of the ClpB/Hsp104 family promote the disaggregation of stress-damaged proteins. The goals of this research will be pursued through the following specific aims: (1) to solve the high-resolution crystal structure of ClpB/Hsp104 using X-ray crystallography, (2) to elucidate the three-dimensional structure of the 0.6-MDa ClpB/Hsp104 assembly by cryo-electron microscopy, and (3) to determine the structural basis by which ClpB/Hsp104 recognizes and binds model substrates. These studies will be complemented by mutational and biochemical experiments to test the hypotheses inferred from these structures. The combination of these approaches will provide a detailed mechanistic understanding of the structure-function relationship of ClpB/Hsp104, and may inspire the design of novel technology that could lead to a potential cure for human prion and amyloid diseases.

**Grant:** 1R01GM067966-01A1  
**Program Director:** PREUSCH, PETER C.  
**Principal Investigator:** O'BRIAN, MARK R  
**Title:** Regulation of Bacterial Heme Biosynthesis  
**Institution:** STATE UNIVERSITY OF NEW YORK AT AMHERST, NY  
BUFFALO  
**Project Period:** 2004/01/01-2007/11/30

**DESCRIPTION** (provided by applicant): Heme is essential for many cellular processes, including oxidative stress responses, detoxification, respiration, signal transduction, and can also serve as a regulatory molecule to affect gene expression. Both the control of heme synthesis and its regulatory function must be reconciled with the fact that heme and its precursors are toxic as free molecules, and iron may be a limiting nutrient. The broad objective of the proposed work is to elucidate the control of bacterial heme biosynthesis, and to reconcile regulatory functions for heme and its precursors with their toxicity in cells. The heme biosynthetic pathway culminates with the insertion of iron into protoporphyrin catalyzed by ferrochelatase. The Irr protein from the bacterium *Bradyrhizobium japonicum* represses the pathway at an early step under iron limitation to prevent protoporphyrin synthesis from exceeding iron availability. Data suggest that Irr interacts directly with ferrochelatase, and responds to iron via the status of heme and protoporphyrin localized at the site of synthesis. In the presence of iron, ferrochelatase inactivates Irr, followed by heme-dependent Irr degradation to derepress the pathway. Under iron limitation, protoporphyrin relieves the inhibition of Irr by ferrochelatase, probably by promoting protein dissociation, allowing genetic repression. The proposal addresses the hypothesis that metabolic control of the heme pathway involves a direct input signal from a biosynthetic enzyme to a regulator to affect gene expression. Furthermore, heme can serve as a signaling molecule without accumulating freely in cells, *irr* gene homologs are found in numerous pathogens and symbionts of eukaryotes, suggesting *lrr*-type regulation in other bacteria. Three specific aims are proposed to address the model. 1. Elucidate the mechanism by which heme promotes iron dependent degradation of Irr. Irr is a conditionally stable protein that involves direct binding of heme to the protein. We propose experiments to address the role of heme with an emphasis on exploring heme-mediated oxidation and proteolysis. 2. Characterize interactions between ferrochelatase and Irr. This interaction allows Irr to respond to the status of heme synthesis and is essential for control of *lrr* degradation and activity. Experiments are designed to further characterize the interaction and to elucidate the molecular basis of the inhibition of *lrr* activity by ferrochelatase. 3. Characterize Irr regulatory function with respect to its DNA-binding properties, functional domains and gene targets.

**Grant:** 1R01GM068025-01A2  
**Program Director:** DEATHERAGE, JAMES F.  
**Principal Investigator:** RAYCHAUDHURI, DEBABRATA PHD  
**Title:** Small Molecule Inhibitors of Bacterial Cell Division  
**Institution:** TUFTS UNIVERSITY BOSTON BOSTON, MA  
**Project Period:** 2004/09/01-2008/08/31

DESCRIPTION (provided by applicant): FtsZ is an essential tubulin-like GTPase that assembles into a ring structure at the site of cell division and recruits other essential division proteins to form the septal ring critical for bacterial cytokinesis. FtsZ is widely conserved in the Bacterial kingdom, including most pathogens and biothreat agents, but is absent in the mitochondria of higher eukaryotes. The essentiality of FtsZ in bacterial cell division, its widespread conservation, its low amino acid identity with tubulin, and its absence in mammalian cells make it an attractive broad-spectrum antibacterial target. Using FtsZ protein-based as well as chemical genetic high throughput screens against small molecule libraries, a number of hits have been identified that cause cell filamentation and bacterial lethality. The whole-cell screens have identified two classes of molecules that cause lethal filamentation with or without affecting FtsZ activity, suggesting inhibition of other essential but as yet unidentified septation targets. One goal of the project is to use these division inhibitors as chemical tools to study septal ring assembly and to identify and validate septation-specific non-FtsZ targets. Another goal is to use the promising inhibitors as chemical scaffolds for analog synthesis and structure-function relationship studies. This effort may aid the development of potent therapeutic leads that target cell division in bacterial pathogens of public health importance and in biothreat agents.

**Grant:** 1R01GM068044-01A1  
**Program Director:** SHAPIRO, BERT I.  
**Principal Investigator:** CORREA, ANA M PHD  
**Title:** Voltage-Gating in Bacterial Ion Channels  
**Institution:** UNIVERSITY OF CALIFORNIA LOS ANGELES LOS ANGELES, CA  
**Project Period:** 2004/04/01-2008/03/31

DESCRIPTION (provided by applicant): Voltage-gated ion channels (VGC) are proteins found in the membranes of practically all cells, that through opening and closing (gating) events let ions flow through between the internal and external milieu of the cells acting as very fast signaling entities. The most characteristic and intriguing aspect of VGC is that their function is modulated by voltage. That means that the protein senses changes in the electrical field and responds by opening, possibly through a sequence of conformational changes. With the advent of high resolution electrical recording techniques combined with the molecular cloning and engineering of ion channel proteins, it has been possible to identify parts of VGC that would serve as voltage-sensors, which has led to proposal of several mechanistic models on how the voltage-sensing event is translated into channel opening. Yet, the molecular and physical natures of the events that take place during voltage-gating are not resolved. It is the long-term goal of this proposal to contribute a physical molecular model of how VGC gate by studying intramolecular distances at rest and while channels are open, using optical tools along with functional recordings. The recent cloning of a bacterial sodium channel, NaChBac, which can be produced in large quantities, purified and reconstituted into lipid membranes, provides a unique opportunity to address these questions in great molecular detail. The specific aims are: 1) Search for regions and residues that undergo distances changes associated with the voltage sensor and between the sensor and the gate region using lanthanide-based resonance energy transfer (LRET) in the reconstituted protein in different conformational states induced by voltage changes in proteoliposomes; 2) Measurement of distances in tandem proteins, purified and reconstituted, bearing a single donor acceptor pair using the same technique as in aim 1; and 3) Functional analysis of voltage sensing and gating using electrophysiology and site directed fluorescence and its correlation to structure and structural changes studied in aims 1 and 2. To measure distances, cysteines are introduced in different parts of the protein and a special sequence, an EF-hand motif that binds lanthanides, is introduced in another part of the same protein. Fluorescent probes are then used to label the cysteine group and are prompted to emit upon excitation of the lanthanide with light. Because groups will be placed in areas suspected to participate in voltage gating, these measurements are expected to contribute real molecular distances and information on molecular rearrangements occurring during voltage gating. VGC are particularly important in nerve and muscle cells because they determine cell excitability and participate in cell-to-cell communication. The results from this work should help in our understanding of a large number of VGC that are crucial in health and in drawing strategies to ameliorate or perhaps eventually cure some illnesses that involve the dysfunction of this important family of channels.



**Grant:** 1R01GM068516-01A1  
**Program Director:** ECKSTRAND, IRENE A.  
**Principal Investigator:** LORY, STEPHEN  
PHD  
MICROBIOLOGY:BACTERIOLOG  
Y  
**Title:** Analysis of *P. aeruginosa* genome diversity and evolution  
**Institution:** HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA  
**Project Period:** 2004/08/01-2008/07/31

**DESCRIPTION** (provided by applicant): *Pseudomonas aeruginosa*, a common inhabitant of water and soil, is also responsible for a range of serious human infections including the chronic respiratory disease of patients with cystic fibrosis (CF). The goal of this proposal is to apply molecular tools to define genetic variations within the genomes of various *P. aeruginosa* isolates. The project will test the hypothesis that unlike most strains of *P. aeruginosa*, the flexible gene pool of strains that are capable of colonizing patients with CF, consists of a specific repertoire of genes encoded within horizontally-acquired genes, often present in genomic islands. Moreover, during the chronic phase of infection these strains accumulate pathoadaptive mutations in the *P. aeruginosa* genome, which allow the bacteria to persist for many years in the respiratory tract. First, using DNA microarrays, analyzing variable segments of DNA by PCR, applying a targeted DNA capture method, and a differential hybridization strategy, we will identify genes in environmental and clinical strains which are not part of the core gene set and therefore may reside in genomic islands. A DNA microarray will be constructed which consist entirely of the genes from this flexible gene pool and it will be used to further analyze the changes in the genomes of a large set of CF isolates, including established lineages of early and late isolates from individual CF patients. The array will be also used to generate a transcriptome of in vivo expressed genes within the CF-specific islands, which will be used to guide the subsequent prioritization of relevant genes. In the second aim, those genomic island-containing genes that are (i) present in great majority of CF isolates, and (ii) expressed in animal models of infection, will be deleted or individual genes will be mutagenized. The effect of these mutations on the virulence of *P. aeruginosa* will be assessed in two animal models of respiratory infection. In the third aim, the appearance of point mutations in the genome will be also monitored in the clones of *P. aeruginosa* isolated over several years from individual CF patients. These mutations may be a required for *P. aeruginosa* to cause a chronic, long-lasting infection in the CF respiratory tract. This hypothesis will be tested in a rat chronic infection model, where accumulation of mutations will be compared to those seen in human infections. The results of the studies proposed in this application should provide new insights into the evolution of a highly successful opportunistic pathogen and shed light on some unexpected virulence mechanisms that function during chronic respiratory tract infections. Moreover, findings from this work should greatly supplement the currently available genomic resources for the research community, particularly those who work with different *P. aeruginosa* isolates. The availability of a "virtual genome", based on complete or partially-completed genome sequences as well as sequences of horizontally-acquired islands, should result in an improved understanding of the interplay between strain specific and core virulence determinants.

**Grant:** 1R01GM068720-01A1  
**Program Director:** RHOADES, MARCUS M.  
**Principal Investigator:** KEILER, KENNETH C MS  
**Title:** The role of tmRNA in development of *C. crescentus*  
**Institution:** PENNSYLVANIA STATE UNIVERSITY-UNIV UNIVERSITY PARK, PA  
PARK  
**Project Period:** 2004/05/01-2009/04/30

**DESCRIPTION** (provided by applicant): Regulation of gene expression by noncoding RNAs (ncRNAs) is an emerging paradigm in biology. ncRNAs regulate a variety of processes, including transcription, translation, RNA modification, mRNA stability, RNA splicing, chromatin structure, and protein stability and activity, in a wide variety of organisms from bacteriophages to humans. Eukaryotic and prokaryotic genomes contain hundreds of uncharacterized ncRNAs, and these genes have been increasingly identified as the loci of mutations that cause developmental defects and several human diseases. One of the most interesting of these ncRNAs is tmRNA, a molecule with properties of both a tRNA and an mRNA, which intervenes in selected translation reactions; tmRNA recognizes selected translation complexes and enters the ribosome to mediate the addition of a peptide tag to the nascent polypeptide, targeting the protein for rapid degradation and releasing the ribosome and mRNA. This unique activity functions both in a translational quality control mechanism and in regulation of gene expression, tmRNA is conserved throughout the bacterial kingdom, and is required for processes such as pathogenesis, symbiosis, stress tolerance, and bacterial development. The long-term objective of this application is to understand the mechanism of action and physiological role of tmRNA in bacteria. This application focuses on the role of tmRNA in the development and cell cycle regulation of *Caulobacter crescentus*. *C. crescentus* is the organism of choice for these studies because the extensive knowledge of the molecular events that control its cell cycle and developmental program provide a unique opportunity to determine the influence of tmRNA activity on cellular differentiation and DNA replication, tmRNA is required for proper timing of DNA replication and the coincident differentiation from swarmer cell to stalked cell in *C. crescentus*. The primary goals of this application are: to identify substrates for tmRNA in *C. crescentus* that are important for control of DNA replication, to determine how tmRNA activity on these substrates affects DNA replication, and to understand how the activity of tmRNA is controlled by the genetic regulatory network of the cell.

**Grant:** 1R01GM068722-01A1  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** MCINTYRE, THOMAS M. PHD BIOCHEMISTRY, OTHER  
**Title:** Leukocyte Responses to Endotoxin  
**Institution:** CLEVELAND CLINIC LERNER COL/MED-CWRU CLEVELAND, OH  
**Project Period:** 2004/08/06-2008/07/31

DESCRIPTION (provided by applicant): Platelet-activating factor (PAF), a phospholipid autocoid, was originally defined as a principle that transferred shock from an animal in anaphylactic shock to a naive animal. It is the most powerful inflammatory lipid mediator yet defined: injection of synthetic PAF causes shock with peripheral hypofusion and vasodilation, pulmonary vasoconstriction, compromises cardiac function, and it induces hemoconcentration with tissue edema. All cellular components of the acute inflammatory/innate immune system express the receptor for PAF. PAF recruits, primes, and activates tissue and elicited macrophages, monocytes, and platelets. PAF has a major role in sepsis and the response to endotoxins. Serum levels of PAF are increased in studies of experimental endotoxemia, and blockade of the PAF receptor completely blocks LPS-induced death. PAF is the major injury-promoting mediator released after inhalation of bacterial endotoxin and injection of PAF acetylhydrolase--a phospholipase that specifically inactivates PAF--prevents death in animal models of anaphylactic shock. Decreased PAF acetylhydrolase activity is associated with clinical sepsis. However, models to the contrary, blockade of the single receptor for PAF has not been efficacious in humans, most likely because PAF receptor antagonists are weak relative to PAF. A more efficacious approach, suppression of PAF synthesis, cannot be accomplished because the rate-limiting enzyme for PAF synthesis has not been molecularly characterized. We do not understand how endotoxin might induce PAF synthesis because we do not know of a cell that makes PAF and releases in response to endotoxin stimulation. There are biologically-active PAF analogs formed by unregulated oxidative attack on circulating lipid, and we do not know whether the PAF activity detected in blood and lung lavage after endotoxin exposure is biosynthetic PAF or a chemically produced oxidized phospholipid that also activates the PAF receptor. We propose to purify and molecularly clone the enzyme that synthesizes PAF, to establish the signal transduction path in leukocytes that connects endotoxin receptors to PAF production, determine if PAF synthesis promotes the formation of PAF-like lipid structural mimetics, and to determine how PAF might affect the cell that synthesizes it. We anticipate this work will lead to a new class of anti-inflammatory agents.

**Grant:** 1R01GM068829-01A2  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** FEIX, JIMMY B PHD  
**Title:** Spin-Labeled Peptide Antibiotics  
**Institution:** MEDICAL COLLEGE OF WISCONSIN MILWAUKEE, WI  
**Project Period:** 2004/09/30-2008/08/31

DESCRIPTION (provided by applicant): Infectious disease remains the leading cause of mortality worldwide. A significant aspect of this problem is the continuing rise of infections that are resistant to most, if not all, conventional antibiotics. To meet this challenge it is essential that new drug targets be identified, and new classes of antibiotics developed. Over the past two decades a large number of naturally-occurring antimicrobial peptides have been found in both vertebrate and invertebrate species that are capable of providing a rapid and broad-spectrum response against a wide variety of pathogens. Because the specificity of these peptides is based on recognition of general properties of the cell membrane the emergence of resistance is exceedingly rare, making them ideal starting points for the development of new antibiotics. A limiting factor in our ability to further enhance the efficacy of these peptides is the lack of detailed knowledge about their mechanism of action, and in particular the manner in which they interact with and disrupt the cell membrane. The goal of this proposal is to develop a clear understanding of peptide-membrane interactions and mechanism of action for a promising and well-defined class of antimicrobial peptides that are synthetic hybrids of the insect peptide cecropin A and the bee-venom peptide, mellitin. Site-directed spin labeling (SDSL) electron paramagnetic resonance (EPR) spectroscopy provides a powerful and well-established approach for the analysis of peptide-membrane interactions that is uniquely suited to providing such a detailed understanding. Specifically, we will use both conventional and pulsed SDSL EPR to measure membrane binding affinities, determine structure, topology, degree of membrane insertion, and peptide-peptide interactions for cecropin-mellitin hybrid peptides in model membranes that mimic both eukaryotic and bacterial membranes and in intact cells. These properties will be related to antibiotic efficacy against a panel of drug-sensitive and drug-resistant bacteria. We will systematically modify peptide composition to define relationships between sequence, membrane interactions, and antibacterial efficacy. Finally, we will synthesize and evaluate the antibiotic efficacy and membrane interactions of peptidomimetic analogs composed of non-natural beta-amino acids. These studies will significantly advance our understanding of the mechanism of action of antimicrobial peptides, and contribute to the further development of peptide and peptidomimetic antibiotics.

**Grant:** 1R01GM068885-01A1  
**Program Director:** OKITA, RICHARD T  
**Principal Investigator:** PELTZ, GARY A  
**Title:** Pharmacogenetic Analysis in Mice  
**Institution:** ROCHE PALO ALTO, LLC PALTO ALTO, CA  
**Project Period:** 2004/05/01-2007/04/30

DESCRIPTION (provided by applicant): The goal of this research project is to demonstrate that genetic information can improve drug treatment regimens. The pharmacokinetic parameters for at least 4 different therapeutic agents will be measured across 11 inbred mouse strains. The strain-specific pharmacokinetic data will be evaluated using a haplotype-based computational method to identify the genetic loci responsible for the variation in pharmacokinetic responses. The computational predictions will be initially evaluated by correlation of allelic and gene expression patterns across inbred mouse strains, and with known information on the metabolism of these drugs. The effect that genetic alterations within the computationally predicted genes have on the metabolism of these drugs will be evaluated using in vitro and in vivo methodology. This experimental system will enable the factors effecting the metabolism of a drug to be efficiently and rapidly identified. Dosing regimens in mice based on information obtained from genotyping key loci will be developed and prospectively tested to evaluate the utility of genetically guided therapeutics. This model murine genetic system will assess the impact of genetically guided dose adjustment within a treatment population. If this approach is successfully applied to human therapy, it would have a significant impact on human health. To enable this, the following will be completed: 1) Polymorphisms in 150 murine genes effecting drug metabolism will be identified. 2) The pharmacokinetic profile (parent and metabolites) of coumadin, bleomycin, isoniazid and ritonavir will be measured in 11 inbred mouse strains. The genetic factors effecting this response will be computationally identified, and the effect that the predicted genes have on the metabolism of these drugs will be analyzed in vitro and in vivo. 3) One drug will be selected for evaluation of the utility of genetically guided dosing, and a clinical trial using genetically guided dosing will be performed in an experimental murine model.

**Grant:** 1R01GM068886-01A1  
**Program Director:** SHAPIRO, BERT I.  
**Principal Investigator:** MUDGETT, MARY B PHD  
**Title:** Dissection of AvrBsT-BST defense pathway in Arabidopsis  
**Institution:** STANFORD UNIVERSITY STANFORD, CA  
**Project Period:** 2004/09/30-2009/08/31

**DESCRIPTION** (provided by applicant): This project will further dissect the molecular and cellular basis of *Xanthomonas campestris* pathovar *vesicatoria* (Xcv) pathogenesis in plants. This bacterium uses a specialized secretory system to orchestrate cell-to-cell communication during the early stages of plant infection. The type III secretion system (TTSS) enables direct secretion and translocation of bacterial proteins into host plant cells. Once inside the host, TTSS effectors, acting presumably as virulence proteins, collectively modulate the cell to initiate disease symptoms in susceptible plants and to activate defense responses in resistant plants. To date, the mechanisms by which XcvTTSS effectors modulate plant physiology are virtually unknown. However, our work has provided important insights to the potential mechanisms used by the YopJ-like effectors, a family that is prevalent in *Xanthomonas* and conserved amongst plant and animal bacterial pathogens. Such conservation implies that pathogenic microbes use a specific mechanism to interfere with host cell signaling. We have shown that Xcv effectors in the YopJ and XopD family function as cysteine proteases inside plant and animal cells. The substrates for these effectors are highly conserved small ubiquitin-like modifiers (SUMO) that are covalently added to a number of regulatory proteins. Thus, we predict that YopJ-like effectors exert their pathogenic effect on host cells by disrupting posttranslational SUMO modification of proteins. The overall goal of this study is to identify and study the plant signal transduction pathways that are affected by the YopJ-like effector AvrBsT during *Xanthomonas* pathogenesis. Specifically, we will: (1) explore the effect of AvrBsT proteolysis on the SUMO pathway in Arabidopsis by isolating SUMOylated plant targets, (2) isolate the Arabidopsis bst disease resistance gene that provides protection against AvrBsT, and (3) use genetic screens to dissect the AvrBsTBST disease resistance signal transduction pathway in Arabidopsis. We believe our findings will reveal common mechanisms used by SUMO proteases conserved in both plant and animal pathogens to control eukaryotic physiology during bacterial-host interactions.

**Grant:** 1R01GM069438-01A1  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** YOUNGER, JOHN G MD  
**Title:** C5a in defense against murine Gram-negative pneumonia  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2004/07/01-2009/06/30

**DESCRIPTION** (provided by applicant): This project is studying how the anaphylatoxin C5a, a protein generated by the immune system upon first interacting with invading microorganisms, helps support host defenses in the lung during acute Gram negative pneumonia. This disease is a serious threat to hospitalized, post-operative, and immunocompromised patients and the bacteria that cause it are increasingly resistant to broad-spectrum antibiotics. Using a murine model of lung infection, our goal is to better define the role of C5a so that improved therapies against Gram-negative pneumonia might be developed. The first aim of the project examines how C5a enhances the in vitro responses of alveolar macrophages to the clinically important pathogen *Pseudomonas aeruginosa* and will measure C5a's effect on phagocytosis, respiratory burst, bacterial killing, and release of proinflammatory mediators. Parallel studies will examine C5a's effect against this pathogen in whole blood. The second aim will study how structures on the surface of Gram-negative bacteria may alter the generation and ultimately the effectiveness of C5a. These studies take advantage of a mutant of a virulent strain of *Klebsiella pneumoniae* in which the gene responsible for initiating synthesis of the surface carbohydrate O-antigen has been deleted. Wild-type and mutant strains will be compared in terms of their ability to promote the generation of C5a and to alter the responses of alveolar macrophages. Parallel studies will be performed using a strain of *E. coli* which has been transformed to synthesize the *Klebsiella* O-antigen to determine if expression of this complement countermeasure can convey virulence to an otherwise nonpathogenic bacteria. The final aim of the proposal will examine 3 strategies to boost the level of C5a produced in the lung during acute infection. These experiments will examine intratracheal therapy with a protein derived from cobra venom which can generate C5a in the absence of an invading pathogen, an inhibitor of the enzyme carboxypeptidase-N (an important deactivator of C5a), and lastly recombinant murine C5a. It is hoped that the results will better define the role of C5a in host defense during Gram-negative lung infection, will increase understanding of how Gram-negative organisms evade complement-mediated lung defenses, and will determine whether novel C5a-enhancing therapies might be used to assist host defense during acute bacterial pneumonia.

**Grant:** 1R01GM069628-01  
**Program Director:** SHAPIRO, BERT I.  
**Principal Investigator:** PHILLIPS, GREGORY J PHD  
**Title:** Functions of 4.5S RNA in the bacterial cell  
**Institution:** IOWA STATE UNIVERSITY AMES, IA  
**Project Period:** 2004/04/01-2008/03/31

DESCRIPTION (provided by applicant): The signal recognition particle (SRP) is a highly evolutionarily conserved ribonucleoprotein complex that functions to target proteins into biological membranes. Because *E. coli* contains a "minimal" SRP, consisting of the Ffh protein in association with a 4.5S RNA species, it provides an attractive system to understand how the translocation machinery functions in all living cells. Understanding the role of the RNA component of the bacterial SRP function is complicated by its essentiality for cell growth and its involvement in protein synthesis. Since few studies have been performed to understand how the sequence determinants of 4.5S RNA contribute to its function in vivo, little is known about why the RNA is an essential component of both the membrane protein localization and protein translation machinery. The long-term goal of our research is to determine how 4.5S RNA functions as a component of the SRP to target proteins to the inner membrane, and in translation by its interaction with elongation factor G. We propose to perform a systematic genetic characterization of 4.5S RNA to determine how the structure of this molecule contributes to its activity, and to probe the function of the RNA in both protein synthesis and membrane protein localization. Our lack of knowledge about the cellular roles of 4.5S RNA is significant in that it prevents us from fully describing the essential processes of protein localization and polypeptide synthesis on a molecular level. Due to the high evolutionary conservation of the SRP RNA, with homologues found in all living species examined to date, we will also better comprehend how this RNA species functions in all living systems. Although recent efforts have been placed largely on in vitro analysis of 4.5S RNA, including biochemical characterization and structural determinations, efforts to understand the function of 4.5 S RNA in vivo has lagged in part due to the lack of genetic systems for studying this molecule. These studies should contribute to improved methods to express and localize membrane proteins of medical importance, as well as lead to the identification of antimicrobial agents.



**Grant:** 1R01GM069680-01A1  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** STASKAWICZ, BRIAN PHD  
**Title:** Molecular Basis of Arabidopsis Innate Immunity  
**Institution:** UNIVERSITY OF CALIFORNIA BERKELEY BERKELEY, CA  
**Project Period:** 2004/09/29-2008/08/31

DESCRIPTION (provided by applicant): Recent studies have shown that both plant and animal bacterial innate immunity share many features in common in response to pathogen infection. The Arabidopsis/Pseudomonas syringae pathosystem is a highly tractable model system to study the molecular basis of bacterial plant innate immunity. The complete genome sequence of both interacting partners has greatly facilitated many molecular approaches including; bioinformatic, genetic, biochemical, cell biological and structural biological studies. A hallmark feature of the bacterial innate immune system in Arabidopsis thaliana is the surveillance of bacterial effector proteins delivered to the plant cell via a type III secretion system by cognate resistance proteins. In our laboratory, we have focused on the RPS2 disease resistance-signaling pathway that specifically recognizes the bacterial effector protein, AvrRpt2. The RPS2 gene encodes for CC/NB/LRR protein and represents a member of one of the subclasses of the "superfamily" of NB/LRR disease resistance proteins found in all plants. The goals of this project are elucidate the molecular events that specify the recognition of bacterial effector proteins and the biochemical and cellular signaling events that determine bacterial innate immunity. In aim 1, we will identify and characterize the amino acid recognition cleavage site of the AvrRpt2 protease. We propose to employ a molecular genetic strategy that will allow us to define and characterize the AvrRpt2 protease cleavage site in AvrRpt2 and RIN4. The identification and characterization of this site will provide insight in the biochemical mechanism of proteolysis and may aid in the identification of addition protein targets in the Arabidopsis genome for this important virulence effector protein. In aim 2, we will purify the AvrRpt2 and RIN4 Proteins for Biochemical Activity Studies. The development of these assays will be critical for the development of the biochemical isolation of the proposed eukaryotic factor that is essential for protease activity described in aim 3. In aim 3, we propose to identify and characterize the putative eukaryotic factor required for AvrRpt2 protease activity by concomitant biochemical and genetic experimental strategies. In aim 4, we will define the molecular basis of RIN4 negative regulation by defining which protein domains of RIN4 are involved in the repression of RPS2 activation and which domains of RPS2 are interacting with RIN4 by utilizing deletion mutagenesis strategies in conjunction with co-immunoprecipitation experiments. Finally in aim 5, we propose to genetically identify mutations that compromise the induction of the Rps2-specified disease resistance Pathway. The genetic dissection of the Rps2 pathway coupled with the identification of RPS2-interacting proteins will put us in a strong position to uncover the molecular events controlling the Rps2 resistance-signaling pathway.

**Grant:** 1R01GM069696-01A1  
**Program Director:** PREUSCH, PETER C.  
**Principal Investigator:** MARONEY, MICHAEL J  
**Title:** Structural Parameters Involved in Metal Recognition  
**Institution:** UNIVERSITY OF MASSACHUSETTS AMHERST AMHERST, MA  
**Project Period:** 2004/09/15-2008/07/31

**DESCRIPTION** (provided by applicant): The mechanisms by which organisms control transition metal ions and the roles of these metals in cellular regulation have emerged as key areas of investigation in metallobiochemistry. Specific metal binding and responses are required by biological systems in order to avoid cross talk between metals in the expression of proteins, in the uptake of specific metals, and for the incorporation of the correct metal into enzyme active sites. The details of how the metalloproteins recognize, bind and respond to the presence of the requisite metal ion is not well established. This is particularly true for transition metal ions, many of which have similar charges and ionic radii. Thus, it seems likely that coordination geometry and ligand preferences (at least among the ligands provided by amino acids) play important roles in distinguishing transition metals. This proposal seeks to use biophysical methods aimed at delineating structural parameters that are involved in determining metal specificity. The overall objective of this research project is to understand the structural parameters that underlie metal specific binding, and the related protein structural responses to specific metal binding, in metalloproteins involved in metal trafficking. Toward this goal, we plan to examine the structural parameters involved in a metalloregulator (NikR), a metallothiostress protein (NikABCDE) and a metallochaperone (HypB)--proteins all involved in nickel trafficking in *E. coli*. The viability of bacteria, including human pathogens, is linked to the acquisition of required metals, and several human diseases have been shown to result from a breakdown in metal trafficking (e.g., Wilson's and Menkes' diseases for copper, genetic hemochromatosis and other hereditary iron overload disorders for iron). A detailed understanding of metal trafficking is thus important to understanding metal metabolism and its effect on human health, and requires an understanding of mechanisms by which metalloregulators, metal transporters and chaperones specific to each required metal operate. In addition, a detailed understanding of the structural parameters involved in metal-trafficking may lead to the design of new antibiotics that interfere with bacterial metal metabolism, which is frequently essential for pathogenesis, and the development of plants that are resistant to metals and useful in bioremediation.

**Grant:** 1R01GM069840-01  
**Program Director:** IKEDA, RICHARD A.  
**Principal Investigator:** GATTI, DOMENICO L MD  
**Title:** Structure, mechanism and evolution of KDO8P synthase  
**Institution:** WAYNE STATE UNIVERSITY DETROIT, MI  
**Project Period:** 2004/03/01-2008/02/28

DESCRIPTION (provided by applicant): The lipopolysaccharide (LPS) of Gram negative bacteria, also known as endotoxin, is responsible for the pathophysiological phenomena of the shock syndrome associated with Gram negative sepsis. 3-Deoxy-Dmanno-octulosonate (KDO) is an essential constituent of the LPS of all Gram negative bacteria. This eight carbon sugar is the first component of the oligosaccharide core region that links the lipid A moiety of LPS to the O-antigen. The combination of lipid A and two KDO units is the LPS component where the endotoxin activity is located. The first step in the synthesis of KDO is the condensation of arabinose 5-phosphate (A5P) and phosphoenolpyruvate (PEP) to form KDO 8-phosphate (KDO8P), the phosphorylated precursor of KDO, catalyzed by KDO8P synthase (KDO8PS). Since LPS is essential for bacterial growth, this enzyme is a potential target for new antimicrobial drugs. There are two classes of highly homologous KDO8PS's differing primarily in the requirement, or lack thereof, of a metal ion for activity. We have determined the structure of one member of the metal-free class, the *Escherichia coli* enzyme, and of one member of the metal-requiring class, the enzyme from the hyperthermophile *Aquifex aeolicus*. These studies have revealed that KDO8PS is a homotetramer with two active sites located on each face of the enzyme. There is alternation of catalysis between the two faces of the enzyme, such that when PEP and A5P bind and react in the active sites located on one face, only PEP binds at the active sites located on the other face. Despite the wealth of information derived from the initial structural and biochemical studies, the mechanism of the reaction catalyzed by KDO8PS is not clear yet. The current project will employ methods of kinetic analysis, spectroscopy, rational mutagenesis, directed evolution and X-ray diffraction to investigate (a) the function of individual residues and active site water in catalysis, and the mechanistic differences between metallo and non-metallo synthases, (b) the molecular mechanism of alternating face catalysis.

**Grant:** 1R01GM069845-01A1  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** HASSETT, DANIEL J. PHD  
**Title:** Role of OxyR in *P. aeruginosa* Biofilm Resistance to H2O2  
**Institution:** UNIVERSITY OF CINCINNATI CINCINNATI, OH  
**Project Period:** 2004/08/01-2008/07/31

DESCRIPTION (provided by applicant): In cystic fibrosis (CF) airway disease, there is compelling evidence for two distinct clinical stages, an oxidative phase (early-stage CF) and an anaerobic phase (chronic, late-stage CF). Within the thick mucus lining the CF airways, the bacteria grow as a "biofilm," a form of development that affords organisms the luxury of enhanced resistance to antibiotics and biocides. The early oxidative phase is based upon a rapid and dramatic influx of neutrophils to the upper airways, an event triggered by bacterial infection. When stimulated, these professional phagocytes mount a potent "respiratory burst," an antimicrobial product from which is hydrogen peroxide (H2O2). In fact, neutrophils can generate millimolar levels of H2O2 within the phagolysosomal vacuole. In contrast, H2O2 levels in blood are nearly 1000-fold lower. Surprisingly, a mutant of the major CF pathogen, *Pseudomonas aeruginosa*, lacking the H2O2-responsive transactivator, OxyR, is exquisitely sensitive to H2O2 and the bacteria perish even in the presence of blood H2O2 levels (micromolar range). Were OxyR to be compromised during human infection, bacteria would be unable to elicit a systemic infection because they would die via H2O2-mediated killing. Therefore, the goal of this proposal is to determine if OxyR of *P. aeruginosa* could serve as a drug target during various *P. aeruginosa* infections. The goals of this proposal are to (i) define a role for OxyR in animal virulence and resistance to human neutrophils, (ii) define the lesions that evoke exquisite sensitivity to H2O2 in the OxyR mutant and what OxyR-controlled gene products contribute to maximal or minimal protection, and (iii) determine whether OxyR is critical for survival of biofilm bacteria to H2O2 and aminoglycosides.

**Grant:** 1R01GM069861-01A1  
**Program Director:** SOMERS, SCOTT D.  
**Principal Investigator:** SPOLARICS, ZOLTAN MD  
**Title:** Erythrocytes, immuno-modulation and G6PD deficiency  
**Institution:** UNIV OF MED/DENT NJ NEWARK NEWARK, NJ  
**Project Period:** 2004/09/01-2008/08/31

**DESCRIPTION** (provided by applicant): It is becoming well appreciated that genetic polymorphism has a major impact on the inflammatory response. Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common known human genetic polymorphism. G6PD deficiency has a major impact on the host response to trauma and infections and protects against malaria infection. The cellular and biochemical mechanisms responsible for the immuno-modulatory effects of G6PD deficiency are not known. Our general hypothesis is that a compromised antioxidant defense in G6PD deficient erythrocytes and in activated white blood cells alters macrophage activation and subsequent T-cell responses during infections. The studies will employ a septic rodent model (cecal ligation and puncture) in the G6PD deficient mouse. In the context of the most common forms of human G6PD deficiencies, the proposed mouse line is relevant because the residual cellular G6PD activity in the deficient mice is similar to that of the human African, type A- G6PD deficiencies. The proposed hypotheses will test (in general) whether (1) macrophage activation and T lymphocyte polarization are different between G6PD deficient and non-deficient animals. These will be tested in vitro as well as in vivo using the cecal ligation and puncture model. (2) We will also investigate whether the sepsis induced erythrocyte damage is more pronounced in G6PD deficiency. The effects of G6PD deficiency on the sepsis-induced organ inflammatory responses and damage, as well as, mortality will also be tested. Additionally, we will investigate (3) whether antioxidant and anti nitric oxide therapies will be beneficial in G6PD deficiency during sepsis. In view of the well-known early oxidative stress and erythrocyte damage during infections and the importance of macrophage and T-cell responses in the inflammatory response the investigated questions are novel and important and may benefit all trauma patients. Elucidating these questions in the context of G6PD deficiency brings in a highly relevant clinical aspect; furthermore, this also provides a unique opportunity to test the relationship between erythrocyte dysfunction and the modulation of the innate immune response. Elucidation of macrophage and T-cell cytokine responses to infection in G6PD deficiency may also be important in providing a mechanism of malaria protection alternative to currently accepted notions.

**Grant:** 1R01GM069872-01A1  
**Program Director:** WEHRLE, JANNA P.  
**Principal Investigator:** GEORGIU, GEORGE  
**Title:** Genetic Analysis of the Escherichia coli Tat Pathway  
**Institution:** UNIVERSITY OF TEXAS AUSTIN AUSTIN, TX  
**Project Period:** 2004/07/01-2008/06/30

DESCRIPTION (provided by applicant): The Twin Arginine Transporter (Tat) is a recently discovered pathway for protein secretion in bacteria and plants. The overall objective of this proposal is to elucidate the mechanism of protein secretion by the Tat pathway using genetic and biochemical means. A unique feature of this application is the development by the PI of novel high throughput screening methodologies that have greatly aided the genetic dissection of the Tat pathway. The proposed studies are focused on the dissection of some of the key early steps in Tat export: Specific Aim 1 will focus on leader peptide binding and the assembly of the translocon complex: 1.1 The analysis of the features of Tat leader peptides that mediate their recognition by the Tat apparatus and also the mechanism by which a subset of Tat leader peptides avoid misrouting into Sec. 1.2 Genetic identification of the TatC region that is responsible for the binding of the Tat consensus motif within the leader peptide. 1.3 The exploitation of a defective leader peptide and its cognizant TatC suppressor that specifically recognizes only that leader (isolated through the studies in 1.2) for the biochemical analysis of changes in Tat protein oligomerization and the events that lead to the formation of the translocon complex. In studies under Specific Aim 2 we will examine the folding quality control feature of Tat. We will seek to: 2.1 Isolate Tat mutations that disable the folding quality control feature of the pathway to permit the export of unfolded polypeptides. 2.2 Examine the role of TatB, and specifically its cytoplasmic domain which our preliminary studies reveal to have chaperone activity and therefore may be responsible for the binding of unfolded mature polypeptides and 2.3 Investigate in detail, the relationship between folding kinetics of substrate proteins and Tat export competence. Overall, the studies proposed in this application will lead to a significant advance in our understanding of the Tat pathway. The revised application is likely to yield significant and completely unique insights into the Tat pathway that, apart from their mechanistic significance, will be of considerable medical benefit, given the established importance of Tat export in bacterial pathogenicity.

**Grant:** 1R01GM069936-01  
**Program Director:** PREUSCH, PETER C.  
**Principal Investigator:** BARQUERA, BLANCA PHD  
**Title:** Na<sup>+</sup>-pumping NADH:quinone oxidoreductase of *V. cholerae*  
**Institution:** RENSSELAER POLYTECHNIC INSTITUTE TROY, NY  
**Project Period:** 2004/08/03-2009/07/31

**DESCRIPTION** (provided by applicant): The proposed research is focused on the structure and function of the Na<sup>+</sup>-pumping NADH:quinone oxidoreductase (Na<sup>+</sup>-NQR) from *Vibrio cholerae*. This enzyme is the primary gateway for electrons into the aerobic respiratory chain of many marine and pathogenic bacteria. As such it plays a role similar to Complex I of the mitochondrial respiratory chain. However, Na<sup>+</sup>-NQR has no homology to Complex I, and instead of translocating protons, pumps sodium ions across the cell membrane creating a sodium motive force that is used by the cell for metabolic work. Sodium metabolism plays an important role in the adaptation of *Vibrio cholerae* to different environments encountered in its cycle of propagation and infection. Furthermore, Na<sup>+</sup>-NQR has been implicated in the regulation of virulence factors in *Vibrio cholerae*. Our goal is to understand the mechanism by which redox reactions are harnessed to drive the translocation of sodium in Na<sup>+</sup>-NQR. For this, it is important to study both the redox processes and the mechanism of sodium transport. We will use an approach that combines site-directed mutagenesis with kinetics and other biophysical methods. We have developed a recombinant Na<sup>+</sup>-NQR in *Vibrio cholerae*, an organism that is congenial to genetic manipulation and for which the complete genome sequence is known. The recombinant enzyme is easily purified by means of a 6X-histidine-tag. We have already made several site-directed mutants that alter cofactor binding, demonstrating that this is a viable system to address functional questions. We plan to make additional mutants, including ones to target conserved charged and polar amino acid residues, which are likely to be involved in sodium pathways inside the enzyme. In order to design the mutants and to evaluate the results, we will need topological and structural information about the enzyme. To this end we plan to create membrane topology maps by using computer predictions together with reporter-protein fusion experiments. We will also make a strong effort to crystallize Na<sup>+</sup>-NQR, since a 3-dimensional structural model is essential for a molecular-level understanding of the mechanism of the enzyme.

**Grant:** 1R01GM069970-01A1

**Program Director:** OKITA, RICHARD T

**Principal Investigator:** WATERMAN, MICHAEL R PHD  
BIOCHEMISTRY:BIOCHEMISTR  
Y-UNSPEC

**Title:** S. coelicolor P450s: Structure/Function/Engineering

**Institution:** VANDERBILT UNIVERSITY NASHVILLE, TN

**Project Period:** 2004/07/01-2008/06/30

DESCRIPTION (provided by applicant): Streptomycetes are bacteria known to inhabit the soil and marine ecosystems worldwide. Within their niches streptomycetes have developed complex and efficient mechanisms of defense against other organisms through the production of anti-bacterial, anti-fungal and anti-nematode compounds and other bioactive compounds, many of which have value in human and veterinary medicine. Streptomycetes also exhibit a broad-based ability to detoxify organic poisons. Both these defense systems involve monooxygenases of the cytochrome P450 (CYP) superfamily representing about 0.25% of streptomycete genes. Many of the CYP gene families overlap between Streptomyces spp. as judged by genomic data, but different subfamilies and families are apparent that reflect the different needs in secondary metabolism. The diversity of P450s reflects the selective pressures allowing subtly different activities to evolve and be retained and a long term goal of this project is to understand general features of substrate-binding to streptomycete P450s and strategies used in different species to redesign P450 primary sequence leading to different natural products. This information will establish a knowledge base from which we can consider the production of new and potent bioactive compounds. The genome of Streptomyces coelicolor A3 (2) contains 18 CYP genes and this current application proposes to establish the function of eight of these CYPs selected from our lead-up work, to correlate their high resolution X-ray structure with function, and to generate modified forms of these CYPs by mutagenesis to examine altered biological activity. S. coelicolor is a particularly good system for this study because a large number of its secondary metabolites are known and they represent a diverse group of organic molecules, but also due to the facile molecular systems established for study of this species. Further, most of the CYPs fall into small groups of related enzymes (same family or subfamily). Three laboratories with overlapping expertise and ongoing collaborations have joined forces to achieve these goals. When complete, this study of CYPs from S. coelicolor will set the stage for alteration of other Streptomyces spp. which through modified defense systems can produce new antibiotics and other drugs for human and animal medicine.



**Grant:** 1R01GM070473-01  
**Program Director:** PREUSCH, PETER C.  
**Principal Investigator:** ROSENZWEIG, AMY C PHD  
**Title:** Particulate Methane Monooxygenase  
**Institution:** NORTHWESTERN UNIVERSITY EVANSTON, IL  
**Project Period:** 2004/05/01-2008/04/30

DESCRIPTION (provided by applicant): Methanotrophic bacteria utilize methane as their sole source of carbon and energy. In the first step of their metabolic pathway, methane monooxygenase (MMO) enzyme systems oxidize methane to methanol. All methanotrophs produce a membrane-bound, copper-containing enzyme called particulate methane monooxygenase (pMMO). Although pMMO is the predominant methane oxidation catalyst in nature, it has proved difficult to isolate, and most investigators have instead opted to study soluble methane monooxygenase (sMMO), a diiron carboxylate-bridged enzyme that is more tractable, but less universal, than pMMO. The structure and mechanism of pMMO remains one of the major unsolved problems in bioinorganic chemistry. In addition to methane, pMMO oxidizes a variety of alkanes and alkenes, including trichloroethylene (TCE), a toxic and carcinogenic groundwater pollutant. Therefore, methanotrophs are excellent candidates for bioremediation of contaminated soil and drinking water. Since pMMO is the primary biocatalytic system in these processes, an understanding of its structure and mechanism could help to engineer microorganisms with different substrate selectivities for future bioremediation applications. Methanotrophs also play a key role in the global methane cycle, preventing large quantities of methane from reaching the atmosphere where it is a potent greenhouse gas. Global warming due to increasing greenhouse gas emissions can have detrimental effects on human health. Some biochemical and biophysical studies of pMMO have been conducted, but most questions regarding the molecular structure and active site remain unanswered. The evidence to date points to a primary catalytic role for copper ions as well as the possible existence of a functional iron center. The exact stoichiometry, location, and ligand environment of the metal ions in the pMMO complex have not been elucidated. The specific aims of the proposed research are to solubilize and purify active pMMO from multiple strains of methanotrophic bacteria, to characterize the metal centers in pMMO, to obtain well diffracting crystals of pMMO, and to determine its X-ray structure.

**Grant:** 1R01GM070642-01  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** KLUG, CANDICE S PHD OTHER AREAS  
**Title:** Spin labeling of MsbA  
**Institution:** MEDICAL COLLEGE OF WISCONSIN MILWAUKEE, WI  
**Project Period:** 2004/05/01-2008/04/30

DESCRIPTION (provided by applicant): The class of proteins termed ATP-binding cassette (ABC) transporters is one of the largest found in nature. Their ability, or lack thereof, to move a variety of ligands across a membrane bilayer using energy from ATP is fundamentally important to bacterial physiology and an array of human pathologies. ABC transporters mediate both the import and export of a wide variety of solutes including antibiotics, lipids, chemotherapy agents, sugars, amino acids, salts and metals. MsbA is the ABC transporter for lipid A that is found in the inner membranes of Gram-negative bacteria such as *Escherichia coli*. Without MsbA present, bacterial cells accumulate a toxic amount of lipid A, which is an essential component of the outer surface of the cell, within their inner membranes. A crystal structure of MsbA was recently obtained that provides an excellent starting point for structural and functional dynamics studies. Although a structure of MsbA is now available, many questions remain concerning its mechanism of transport. The goal of this proposal is to elucidate the conformational dynamics that occur in MsbA, a bacterial ABC transporter, upon binding ATP in its nucleotide binding domain and upon recognition and transport of lipid substrates in its helical core, utilizing site-directed spin labeling (SDSL) electron paramagnetic resonance (EPR) spectroscopy techniques. In order to address the proposal that the MsbA homodimer undergoes significant conformational rearrangements upon ATP and lipid binding that are essential to its function as a lipid exporter, the following points will be addressed: 1) evaluate the quaternary structure of MsbA reconstituted into lipid membranes; 2) investigate the conformational dynamics of the MsbA dimer upon ATP binding; and 3) investigate the conformational dynamics of the MsbA dimer upon lipid binding. It is anticipated that these studies will produce valuable insights into the local and global structural dynamics of MsbA as it functions in its role as a lipid transporter.

**Grant:** 1R01GM070768-01  
**Program Director:** DEATHERAGE, JAMES F.  
**Principal Investigator:** EHRENBERG, MANS PHD  
**Title:** Biochemical and Structural Analysis of the Ribosome  
**Institution:** UPPSALA UNIVERSITY UPPSALA,  
**Project Period:** 2004/05/01-2008/04/30

**DESCRIPTION** (provided by applicant): The ultimate aim of the project is to understand the structural rearrangements of the ribosome that underlie the different phases of protein synthesis in bacteria and yeast. To achieve this goal, functional and structural analyses will be fully integrated to maximize synergisms between the different competence areas of the participating groups. In detail, the project aims (1) To clarify how initiation of protein synthesis takes place with the help of the three initiation factors IF1, IF2 (a G protein) and IF3. (2) To study how translocation of tRNAs occurs on the eubacterial ribosome with the help of elongation factor EF-G (a G protein). (3) To study the solution structure of eubacterial class-1 release factors to decide if it is similar to the crystal structure of RF2. (4) To clarify the role of domain 1 in eubacterial release factors and the role of protein L11 in the large ribosomal subunit in termination of protein synthesis. (5) To clarify the mechanism behind ribosomal recycling, catalysed by EF-G and ribosomal recycling factor RRF, back to a new round of initiation. (6) To clarify the role of peptide release factor eRF3 (a G protein) in eukaryotic protein synthesis. All experiments are based on in vitro systems with components of high purity from E. coli and yeast. The approach is to integrate functional assay carried out with biochemical tools, including fast kinetics methods (quench-flow, stopped flow) with structural analysis carried out mainly with cryo-electron microscopy but also with low angle X-ray scattering. Ribosomal complexes will be shipped from Uppsala to Albany and prepared under controlled temperature conditions for cryo-EM studies, including time resolved cryo-EM when applicable. Grids will be imaged with a liquid nitrogen or liquid helium cooled cryo-EM microscope, and the images will be analyzed with the SPIDER image processing system. Between 30,000 and 100,000 images will be collected for 10-12A resolution. The density maps will be analyzed by manual or automated fitting of x-ray structures or by real space refinement to obtain atomic models.

**Grant:** 1R01GM070873-01  
**Program Director:** LEWIS, CATHERINE D.  
**Principal Investigator:** KISKER, CAROLINE PHD  
**Title:** Nucleotide Excision Repair: From Recognition to Incision  
**Institution:** STATE UNIVERSITY NEW YORK STONY BROOK STONY BROOK, NY  
**Project Period:** 2004/04/01-2008/03/31

DESCRIPTION (provided by applicant): Maintenance of the correct genetic information is crucial for all living organisms. Mutations are the primary cause of hereditary diseases, as well as cancer, and may also be involved in aging. 80 to 90% of all human cancers are ultimately due to DNA damage. Different repair mechanisms have evolved to protect the genome. Nucleotide excision repair (NER) is well known for the removal of bulky DNA lesions and is unique in its versatility to repair a broad substrate range of DNA lesions. In humans, NER is the major repair mechanism to protect DNA from damage induced by ultraviolet light. The phenotypic consequences of defective genes involved in NER are apparent in three severe diseases: xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy. The overall goals of this project are to understand the fundamental mechanisms of nucleotide excision repair by a series of studies involving the bacterial UvrABC NER machinery. In these studies specific proteins and complexes involved in nucleotide excision repair will be characterized by a combination of biochemical, crystallographic and molecular biology experiments. The studies described in this project will verify three hypotheses: (1) The bacterial NER protein UvrB recognizes damage by intercalating a b-hairpin between the DNA duplex and forms tight interactions with the undamaged strand only after formation of the pre-incision complex. (2) The lesion containing strand is mainly held in place by base stacking interactions and is freely accessible for recognition by UvrC, the endonuclease, which is responsible for the excision process. (3) A conformational change takes place in either UvrB and/or UvrC to allow the sequential process in which 3' incision precedes 5' incision. The proposed studies are divided into four specific aims: (1) Characterization of the UvrA/UvrB interaction and identification of DNA binding sites on UvrB. (2) The pre-incision complex: Structural characterization of the UvrB.DNA pre-incision complex. (3) Characterization of UvrC in the absence of UvrB and DNA: Determination of the three-dimensional structure of UvrC prior to binding to UvrB and DNA. (4) Analysis of the UvrB.UvrC.DNA complex: The proposed studies will delineate the roles of the individual proteins and their complexes formed in the process of NER.

**Grant:** 1R01GM071601-01  
**Program Director:** RODEWALD, RICHARD D.  
**Principal Investigator:** YANG, ZHAOMIN BS  
**Title:** Regulation and Mechanism of *M. xanthus* Social Gliding  
**Institution:** VIRGINIA POLYTECHNIC INST AND ST UNIV BLACKSBURG, VA  
**Project Period:** 2004/08/01-2009/07/31

DESCRIPTION (provided by applicant): Type IV pill (Tfp) are important determinants of bacterial virulence and biofilm formation which is the cause of many opportunistic and chronic bacterial infections. Tfp also mediate a form of bacterial surface motility known as social (S) gliding in *Myxococcus xanthus* and twitching in other bacterial species. The function of Tfp in surface motility correlates with their function in bacterial pathogenicity and biofilm formation. Mutations in genes essential for Tfp biogenesis and function lead to simultaneous defects in bacterial virulence, biofilm formation and Tfp-mediated motility. Our long term goal is to use *M. xanthus* S-motility as a model to study Tfp-mediated functions and signaling. S-motility itself also warrants further studies on its own merit because it is important to the developmental process of *M. xanthus* multicellular fruiting bodies. Besides Tfp, *M. xanthus* S-motility requires another cell surface component known as extracellular fibrils. Previous studies showed that the *dif* genes, encoding homologues of bacterial chemotaxis proteins, are central to the biogenesis or production of fibrils. Recent findings suggest that the *pil* genes which are required for Tfp biogenesis and function are also involved in the regulation of fibril biogenesis in *M. xanthus*. We propose to do the following. First, we will verify the requirement of fibril biogenesis for Tfp. We will further construct double mutants to examine the epistatic relationships in the regulation of fibril biogenesis among *dif* and *pil* genes as predicted by our hypothesis. Second, we will use yeast two-hybrid (Y2H) system and phosphorylation studies to examine if the Dif chemosensory-like proteins interact with one another physically and biochemically as their counterparts in bacterial chemotaxis and to identify any novel interactions that may exist among Dif. Third, it is unknown whether DifA, a homologue of methyl-accepting chemoreceptor proteins (MCP), localizes to specific subcellular regions and how DifA and the Dif pathway respond to stimulation. We will study DifA localization and the dependence of correct DifA localization on *dif* and *pil* genes by immunofluorescence microscopy. We will additionally construct and use NarX-DifA fusions to examine DifA modification and the signaling properties of the Dif pathway in response to stimulation by nitrate. Finally, Y2H and genetic screens will be carried out to identify new genes important for fibril regulation and biogenesis, especially downstream of Dif proteins.

**Grant:** 1R01GM071962-01  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** COLLADO-VIDES, JULIO PHD  
**Title:** Gene Regulation: E.coli Database Integrated Modeling  
**Institution:** UNIVERSIDAD NACIONAL AUTONOMA DE CUERNAVACA MORELOS,  
MEXICO  
**Project Period:** 2004/08/01-2008/07/31

**DESCRIPTION** (provided by applicant): A major challenge in post-genomic biology is the understanding of the complex molecular internal machinery of the cell. A fundamental component in the machinery to take decisions is the regulatory network of transcription initiation. The purpose of this application is to enhance the major annotation project on transcriptional regulation and operon organization of the model organism *Escherichia coli* K-12. This project is a conceptual continuation and expansion of a previous grant supporting the annotation work enriching RegulonDB. In addition to continuing literature curation, we propose to perform an exhaustive mapping of all possible missing promoter sites in the genome, as well as to perform several theoretical analyses of the regulatory network, together with simulations and comparisons with microarray data performed in other laboratories. We plan to complement literature knowledge with computational predictions, and to actively search for the missing links that will permit us to put together transcription, transport, signal transduction and metabolism, taking advantage of several existing databases. We will determine how much this cellular knowledge is consistent with the large amounts of microarray experiments performed in several laboratories. These comparisons require building several computational and chemical models of regulatory simple and complex interactions determining gene expression, as well as their consequences in the overall cellular network (transport, signal transduction and metabolism). We will analyze the network in modules, motifs, and maps, using different mathematical criteria based on the statistical properties of the network connectivity, as well as on biological knowledge of conditions and microarray experiments. Finally, we intend to perform low-resolution discrete simulations of the dynamics of the network, as well as detailed stochastic modeling of selected maps and subcomponents of the network. Modeling and its comparison with experiments shall enhance the overall quality and depth of the classical and active annotation of the regulatory network of the best known and studied single cell organism. Having a better understanding of a whole cell, shall provide an important contribution to basic biology and thus to the foundations to understand health and disease.

**Grant:** 1R01GM072004-01  
**Program Director:** WHITMARSH, JOHN  
**Principal Investigator:** WOLGEMUTH, CHARLES W PHD  
**Title:** Elastic Model of Spirochete Morphology and Motility  
**Institution:** UNIVERSITY OF CONNECTICUT SCH OF FARMINGTON, CT  
MED/DNT  
**Project Period:** 2004/05/01-2008/04/30

DESCRIPTION (provided by applicant): Polymer filaments play a major role in bacterial motility and morphology. Spirochetes, bacteria that swim by rotating helical flagella that are encased within a periplasmic space (the space between the inner and outer cell membranes where the cell wall material is located), are excellent model systems to study how elastic polymer filaments coupled to a bacterial cell wall can affect motility and morphology. Rotation of the flagella induces rotation and deformations in the cell wall. Because the flagella remain within the periplasm, the elasticity of the helical flagella competes with the preferred morphology of the cell wall. How this interplay between flagella and wall elasticity leads to total cell morphology and how forces developed by the flagellar motor drive motility is poorly understood. This grant will support the broadening of elastohydrodynamics to handle systems in which two or more elastic filaments are coupled together. This new mathematical framework will then be employed to model the morphology and motility of spirochete bacteria. Through study of the static shapes predicted by this model, this work will test the experimentally justified hypothesis that the flagella and cell wall are the dominant morphological determinants in spirochete bacteria and explore how the number and length of the periplasmic flagella affect these shapes. A detailed analysis of how this system responds to external forces and torques, as would be produced by the flagellar motor, will lead to an understanding of the dynamic shape changes that spirochetes undergo while swimming. Finally, the coupling of the resulting cell conformations to the viscous fluid environment surrounding them will be investigated to quantify the propulsive force generated by the rotation of the flagellar motor for many spirochetes and also explain the precession of the flat wave morphology in *Borrelia burgdorferi*. This grant will also support the experimental measurement of the bending moduli of the cell wall and the periplasmic flagella.

**Grant:** 1R01GM072085-01  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** GIRVIN, MARK E PHD  
**Title:** Structural Analysis of Multidrug Transport  
**Institution:** YESHIVA UNIVERSITY BRONX, NY  
**Project Period:** 2004/08/01-2008/07/31

DESCRIPTION (provided by applicant): Bacteria have developed several methods to resist the lethal effects of antibiotics. The broadest spectrum resistance results from the action of Multidrug resistant pumps (MDRs), which extrude a range of compounds of quite diverse chemical structure. The Small Multidrug Resistance pumps (SMRs) are 100- 110 residue dimeric proton-drug antiporters that contain the full multidrug transport machinery, stripped to its barest essentials. Hence they are ideal transporters for a comprehensive structural and functional understanding of drug transport and inhibition in a medically important MDR. We propose to determine the structures of the conformations making up the functional cycle of an SMR, and identify the binding determinants for multiple drugs and inhibitors using solution NMR by: 1) Determining the structure of a dimeric SMR in lysolipid micelles in its protonated state, 2) Measuring the affinities of multiple drugs and inhibitors above and below the pKa of the glutamate essential for transport, 3) Identifying the binding determinants for inhibitors and transportable drugs, and 4) Determining the conformational changes induced by substrate and inhibitor binding, and by deprotonation of the critical glutamate. In addition to the MDR pumps, membrane proteins are responsible for transmembrane signaling, energy transduction, and ion and metabolite transport. These proteins are important in infectious disease, genetic disorders, and cancer. Despite their importance, and the need for structure to understand their function, relatively few such structures are available. For transporters and receptors, ligand binding and transport involve multiple conformations and dynamic changes. Solution NMR is an ideal method for examining these processes. Firmly establishing NMR methods to study larger helical membrane proteins - here a dimer with total of eight transmembrane helices, will have as long-term an impact as the specific findings for an SMR.



**Grant:** 2R15GM063637-02  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** BRABBAN, ANDREW DAVID PHD  
**Title:** Bacteriophages for Food-Chain Biocontrol Of E. coli O157  
**Institution:** EVERGREEN STATE COLLEGE OLYMPIA, WA  
**Project Period:** 2001/08/01-2006/03/31

DESCRIPTION (provided by applicant): In January 2003 the leading 200 beef industry companies held an unprecedented meeting to develop a "unified battle plan" to eliminate E. coli O157 from their industry using methods based in sound science. This important food-borne pathogen causes >70,000 illnesses yearly in the U.S., >75% of them directly linked to livestock; at present 28% of U.S. cattle carry O157, though they show no symptoms. Preliminary experiments supported by our current NIH/AREA grant suggest that, with targeted research and development, bacteriophages could play a significant role in protecting our food supply. We isolated CEV1, the T4-like phage we have used most, from a flock of sheep found to be highly resistant to gut colonization by O157 during probiotic studies at the Southern Plains USDA/ARS Research Center, our collaborators on this project. We have since isolated promising phages from two other flocks of sheep. The long-term objectives of this proposal are to (1) isolate and characterize further phages capable of infecting E. coli O157:H7, (2) investigate the dynamics and physiology of replication of those phages under conditions relevant to the natural environment and to potential therapeutic applications, i. e. during anaerobic respirative and fermentative growth of the host, and select the most promising phages and multiphage cocktails; and (3) use appropriately-designed mixed-culture fermenters and experiments in sheep to assess the potential for using these phages to reduce the O157:H7 load in the guts of livestock and thus in the foodchain. Such experiments are very well suited to work by our undergraduate students, helping them develop a firm foundation in microbial ecology and physiology, molecular biology, general microbial and anaerobic techniques, and experimental design, along with the satisfaction of contributing significant new research results. In preliminary studies, O157:H7 was successfully eradicated from gut models by CEV1 treatment in 12 days and O157 levels were markedly reduced in sheep in two days by CEV1 and CEV2.

**Grant:** 1R15GM069394-01  
**Program Director:** ANDERSON, RICHARD A.  
**Principal Investigator:** WHIPPLE, FREDERICK W PHD  
**Title:** Polymerization and Activation of Bacterial RecA Proteins  
**Institution:** CALIFORNIA STATE UNIVERSITY FULLERTON FULLERTON, CA  
**Project Period:** 2004/01/01-2006/12/31

DESCRIPTION (provided by applicant): Bacterial genetic techniques will be used to investigate the mechanism by which molecules of the RecA protein of the bacterium *Escherichia coli* polymerize around DNA to form an active nucleoprotein complex. Proper formation of this complex is essential to the processes of DNA repair and DNA recombination, both of which are necessary for survival. RecA is closely related to similar proteins found in cells of higher organisms, including humans. This work is relevant to human health because defects in DNA recombination and repair in humans usually lead to cancer or severe genetic disorders. A deeper understanding of these processes may help in the development of treatments and diagnostic techniques for these specific illnesses. The genetic technique of mutant-suppressor pair analysis will be applied to this problem. This involves the targeting of a mutation to one member of a pair of interacting proteins, and then seeking second mutations in the other member of the pair that reverse the effect of the first mutation. Mutant pairs such as this are very useful in mapping individual amino acid contact points between interacting proteins. To implement this strategy, an altered form of the RecA polymer will first be generated. This will be done by obtaining pair of recA mutants that are each unable to polymerize by themselves, but are able to interact with each other to form fully functional polymers. These polymers will be characterized by alternating interface specificities.

**Grant:** 1R15GM069398-01  
**Program Director:** FLICKER, PAULA F.  
**Principal Investigator:** WILLEY, JOANNE M PHD  
**Title:** Cell-cell communication in *Streptomyces coelicolor*  
**Institution:** HOFSTRA UNIVERSITY HEMPSTEAD, NY  
**Project Period:** 2004/02/01-2007/01/31

DESCRIPTION (provided by applicant): Extracellular communication among bacteria involves a rich diversity of signaling molecules, signal reception and transduction systems. The exchange of diffusible signaling molecules between prokaryotes is important in a variety of physiological processes including development, pathogenicity, biofilm formation and competence. The proposed research program is designed to explore the structure and function of extracellular signaling molecules involved in the morphological differentiation of the filamentous microbe *Streptomyces coelicolor*. *S. coelicolor* is a model streptomycete; this genus is responsible for the production of approximately 70% of the antibiotics currently prescribed, as well antihelminthic and immunosuppressive agents and drugs with antineoplastic activity. The exchange of signaling molecules among *S. coelicolor* cells is evidenced by the complete rescue of development when two *S. coelicolor* bald (bld) mutants blocked in morphogenesis are plated near one another. Based on the pattern of complementation when bld mutants are plated in pair wise combination, it is hypothesized that a hierarchical signaling cascade occurs such that each bld gene is involved directly or indirectly in the synthesis, perception of, or response to one of six different extracellular signaling molecules. Each signal then induces the production of the next, ultimately leading to synthesis of the structural morphogen, SapB. The specific aims of the proposed research are to purify and resolve the structure of three of these bld-derived signaling molecules. Purified signals will then be used to probe the role of these molecules in initiating morphological differentiation. Antibodies will be prepared too so that the spatial, temporal and genetic regulation of signal production can be evaluated and microarray analysis will be performed to identify genes whose expression responds to the presence of the extracellular signals. Finally, as an Academic Research Enhancement Award (AREA) proposal, the program of proposed research will serve to train undergraduate and Master's level students in the design and execution of experiments in the life sciences.

**Grant:** 1R15GM070562-01  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** VARELA, MANUEL F PHD  
**Title:** Substrate Selection in Homologous Bacterial Transporters  
**Institution:** EASTERN NEW MEXICO UNIVERSITY PORTALES, NM  
PORTALES  
**Project Period:** 2004/07/01-2007/06/30

**DESCRIPTION** (provided by applicant): Solute transporters are defective in genetic diseases and confer bacterial and cancer drug resistance. Transporters have substrate specificity, which is poorly understood, thus hindering our understanding of transport. It is unclear whether amino acids that confer sugar specificity in the lactose permease of *E. coli* (LacY) also confer sugar selection in homologous transporters for melibiose (MelY), raffinose (RafB) and sucrose (CseB). Until solute specificity is understood in homologous transporters, it will be difficult to know how transport is accomplished. The long range goal is to understand the molecular basis of substrate recognition during transport. The specific objective of this research is to determine whether (the molecular properties) the solute binding sites in homologous solute transporters differ in their amino acids that determine specificities. The central hypothesis is that functional differences between transporters (i.e., solute specificities) are dictated by subtle differences in sequence (and thus, structure), since the seemingly diverse transporters share related amino acid sequences. The rationale for the proposed research is that once it is known whether homologous sugar transporters mediate substrate specificity by similar or distinct binding and transport properties, we will know whether a common molecular basis exists for transport in seemingly diverse transporters, and the potential will exist for engineering transporters to translocate highly desirable solutes for therapeutic purposes. The solute specificity profiles for MelY, RafB and CscB homologues are distinct and thus make our experimental system unique for a comparative study of substrate specificity. The specific aims are independent of the completion of one another. Specific Aim 1: Identify amino acid residues that determine sucrose transport in MelY. We propose to isolate and characterize mutants containing MelY that transport sucrose. In preliminary work, we have found several sucrose fermentation positive mutants. We have published data showing mutations in helices 3 and 6 of MelY that transport maltose. Specific Aim 2: Identify amino acids that determine sucrose and maltose transport in RafB. We propose to isolate and characterize RafB mutants that transport sucrose. We isolated maltose positive mutants; we will study maltose uptake and accumulation in these mutants using transport assays. We have preliminary data showing that RafB has distinct binding sites for raffinose and lactose. Specific Aim 3: Identify residues that determine melibiose and maltose transport in CscB. We will isolate cells with mutated CscB that transport melibiose or maltose, and then study transport. We have found maltose positive mutants. We have preliminary data showing that CscB transports lactose. The comparative nature of the study of solute specificity in homologous transporters is innovative, as most other studies focus on solute binding and transport of naturally occurring substrates. Data generated by the proposed work will be innovative because MelY, RafB and CscB are homologous to many transporters, and knowledge of solute specificity mechanisms gained here will be applicable to transporters involved in genetic diseases and to infectious disease-causing bacteria and their drug resistances.

**Grant:** 1R15GM070563-01  
**Program Director:** WOLFE, PAUL B.  
**Principal Investigator:** FOWLER, ROBERT G  
**Title:** Pathway of Spontaneous Mutagenesis in Escherichia coli  
**Institution:** SAN JOSE STATE UNIVERSITY SAN JOSE, CA  
**Project Period:** 2004/04/01-2007/03/31

**DESCRIPTION** (provided by applicant): The long-term objective of this study is to acquire a greater understanding of the mechanisms of spontaneous mutagenesis as they occur in cells. Directly related to the creation of mutations is the fidelity mechanisms that cells employ to keep mutational events at a low level of occurrence. The list of human diseases and disorders that are affected by mutational events is extensive and ranges from the so-called single gene genetic disorders to multifactorial conditions that come about via the combined effects of environmental factors and multiple mutational events that occur to achieve the altered phenotype. Even infectious diseases once thought to be almost exclusively environmental in nature are now known to be influenced by the genotype of both the host and the pathogen. Somatic mutations give rise to the variety of human cancers. While great attention has been given to mutational events caused by environmental chemical mutagens, it is likely that most mutational events that occur in human populations are spontaneous in nature. A direct approach to study mechanisms of spontaneous mutagenesis is to characterize mutant alleles, called antimutator alleles that cause decreases in spontaneous mutation frequencies. This is possible with the genetically well-characterized bacterium, *Escherichia coli*. A series of suspected or partially characterized antimutator alleles will be fully tested and characterized with the goal of revealing mutagenic pathways. Since such pathways and the fidelity mechanisms that keep mutational events at a low frequency are well conserved, it is expected that results obtained from this bacterial study would be applicable to other organisms, including humans. For the past several years, many studies have collectively shown that spontaneous mutations occur in starving or stressed bacterial cells. Some of these mutations are caused by the action of a group of specialized DNA polymerases that are capable of synthesizing past damaged or unusual DNA templates. In *E. coli* it has been shown that one or more of these polymerases may be active in starving/stressed cells where apparently the synthesis past a damaged template comes at a "cost" of creating mutations. The present application intends to more fully characterize the creation of mutations under these physiological conditions. Humans possess a number of these specialized DNA polymerases whose activities may be responsible for a significant portion of the human mutational load.

**Grant:** 1R15GM071009-01  
**Program Director:** CHIN, JEAN  
**Principal Investigator:** GREEN, JACALYN M PHD  
**Title:** Transport and Metabolism of Folate Analogs in E. coli  
**Institution:** MIDWESTERN UNIVERSITY DOWNERS GROVE, IL  
**Project Period:** 2004/05/01-2007/04/30

DESCRIPTION (provided by applicant): Reduced derivatives of folic acid are involved in the biosynthesis of cellular components, including DNA, RNA, and protein. Essential in the human diet, folate is synthesized de novo in microorganisms. Conventional wisdom and early experiments supported the idea that many bacteria, including *Escherichia coli* (E. coli), are unable to import and utilize exogenous folate. This explained why the sulfonamide antibiotics, structural analogs of p-aminobenzoic acid (PABA), effectively inhibited folate biosynthesis and bacterial replication, despite residing in a human host containing folate. We have found that E. coli contains abgT, a cryptic gene for a transport protein that, when expressed in elevated levels, imparts two phenotypes: a highly increased sensitivity to the folate analogs, aminopterin and methotrexate, and the ability for a PABA auxotroph to grow on nanomolar quantities of the folate breakdown product, p-aminobenzoyl glutamate (PABA-GLU). PABA-GLU is a major catabolite of folate in humans, and is excreted in both urine and feces. The identification of AbgT as a possible transporter for either folate or folate catabolites (PABA-GLU) raises the possibility that E. coli possesses the ability to import folate and/or its breakdown products. This may occur in response to growth conditions that have not been identified previously. Folate transport and catabolism have remained largely unrecognized and unstudied in E. coli. This research proposal is designed to characterize this transport system. Specifically, we propose to characterize the transport protein AbgT with regard to its ability to take in aminopterin, methotrexate, folate, and PABA-GLU. We also intend to investigate a range of growth conditions to determine what might induce expression of abgT in wild-type cells; growth conditions to be investigated include nutrient starvation, anaerobic growth, and alterations in media pH. We also plan to purify and characterize the enzyme that cleaves PABA-GLU to form PABA, and to identify the gene associated with this activity. Finally, we plan to construct a strain of E. coli that can grow on physiologic concentrations of exogenous folate. This will likely entail optimizing transport of folate as well as reduction of folate to dihydrofolate. Successful completion of these studies will advance our understanding of basic metabolism and catabolism of the important vitamin folic acid in E. coli, especially with regard to a newly identified transport protein AbgT.

**Grant:** 1R21GM070915-01  
**Program Director:** TOMPKINS, LAURIE  
**Principal Investigator:** HWA, TERRENCE PHD  
**Title:** Combinational Transcription Control in Bacteria  
**Institution:** UNIVERSITY OF CALIFORNIA SAN DIEGO LA JOLLA, CA  
**Project Period:** 2004/04/01-2006/03/31

DESCRIPTION (provided by applicant): The survival and well being of an organism depend crucially on the ability of its cells to coordinate their gene activities in response to a vast number of cellular and environmental signals. This is often accomplished combinatorially through a large number of protein-protein and protein-DNA interactions. A major goal of post-genome biology is to characterize these interactions and decipher the complex regulatory circuits/networks that they define. Instead of directly dissecting the regulatory program of a specific organism, we investigate different molecular strategies an organism could adopt to integrate and regulate signals. The focus of the proposed research is on combinatorial transcription regulation. We propose experiments to implement a number of novel transcriptional regulatory functions involving two well-characterized *E. coli* transcription factors. The regulatory functions will be demonstrated *in vivo*, by quantifying the expression of a reporter gene for different combinations of inducer concentrations. The proposed research is a pilot study aimed at the larger goal of implementing *cis*-regulatory control involving arbitrary regulatory proteins in a cell. Applying this capability to bacteria endowed with special sensors/receptors, one would be able to design *cis*-regulatory sequences to recognize and report unique patterns of detectable traits corresponding to specific chemical pollutants or harmful biological agents. Furthermore, the directed evolution approach can in principle be extended to breeding desired promoter sequences in eukaryotic cells. Such approaches could lead to sensitive, non-invasive cellular reporters of complex transcriptional patterns *in vivo*. The information obtained could be of significant value to the diagnosis and treatment of complex diseases.

**Grant:** 2R37GM060632-05  
**Program Director:** DEATHERAGE, JAMES F.  
**Principal Investigator:** ROTHFIELD, LAWRENCE I MD  
**Title:** Studies of E. coli Min proteins  
**Institution:** UNIVERSITY OF CONNECTICUT SCH OF FARMINGTON, CT  
MED/DNT  
**Project Period:** 2000/02/01-2009/03/31

**DESCRIPTION** (provided by applicant): The long-range goal of this project is to determine the mechanism used by bacteria to select the proper cell division site at midcell. The three Min proteins, MinC, MinD and MinE, are required for proper site selection. The proteins have a unique cellular localization pattern and undergo a unique pole-to-pole oscillatory cycle. We have recently shown that the MinCDE proteins are organized into spiral filaments that wind around the cell between the two poles in a cytoskeletal-like structure that appears distinct from a coiled structure formed by the MreB protein. During the proposed grant period we will use a combination of fluorescence microscopy, biochemistry and genetics to achieve the following aims: i. Min protein structure and function-- To determine the effects of mutations that interfere with the topological specificity function of the MinCDE system, ii. Long-range organization of the Min system-- To define the organization of the MinDE cytoskeletal-like elements within the cell, to determine whether the Min and MreB spiral filaments are separate structures, to characterize proteins that interact with MinD and MinE, and to determine whether there is a relationship between the Min spiral filaments and chromosome segregation, iii. MreB-- To isolate the MreB and Min cytoskeletal-like structures, to characterize proteins that interact with MreB, and to define the behavior of the MreB cytoskeletal-like structures during the course of the cell cycle.



**Grant:** 1U01GM070749-01  
**Program Director:** ANDERSON, JAMES J.  
**Principal Investigator:** LONGINI, IRA M PHD  
**Title:** Containing Bioterrorist and Emerging Infectious Diseases  
**Institution:** EMORY UNIVERSITY ATLANTA, GA  
**Project Period:** 2004/05/01-2009/04/30

DESCRIPTION (provided by applicant): The overall objective of this research is to develop, validate, and implement mathematical models for the transmission and within-host dynamics of bioterrorism agents or naturally occurring infectious diseases. These models will be used to assess the effectiveness and efficacy of various interventions to aid the distribution and allocation of resources in response to such outbreaks. Specific aim 1 is to develop epidemic simulation models for the transmission of infectious diseases in question: a. to develop stochastic epidemic simulation models for a typical American community; b. to use the epidemic simulation models to evaluate the effectiveness of interventions involving surveillance and containment, vaccination, antimicrobials, closing of key institutions, and other control strategies; c. to develop stochastic optimization methods to find the best intervention strategy, constrained by the resources available; d. to adapt the epidemic simulation models for smallpox, pandemic influenza, SARS, and other possible bioterrorism agents or naturally occurring infectious diseases; e. to use the epidemic simulation models to determine the important parameters for infection transmission and to use this information to design field studies and intervention studies; f. to use and develop statistical methods to estimate the important parameters and variables from data. Specific aim 2 is to develop models of the within-host dynamics of pathogens which cause acute infections in vertebrates: a. to construct exploratory models for the interplay between the pathogen and host immune response; b. to refine, to develop further and to test these models of pathogenesis including the use of existing data on lymphocytic choriomeningitis virus (LCMV) and listeria monocytogenes (LM) infections of mice; c. to use the experience from the specific aim 2.b. to extend the models in the specific aim 2.a. to examine the more challenging acute infections of humans including bioterrorism agents or naturally occurring infectious diseases; d. to model development of resistance under selective pressure by antimicrobial and antiviral treatment and prophylaxis by antimicrobials or vaccination; e. to combine the stochastic epidemic simulation models with models for within-host generation of resistance to examine spread of resistance within the host population.

**Grant:** 2R01HD018184-25  
**Program Director:** LOCK, ALLAN  
**Principal Investigator:** WILSON, CHRISTOPHER B MD OTHER CL MED:CLINICAL  
MEDICINE,UNSPEC  
**Title:** HOST DEFENSE TO NEONATAL INTRACELLULAR PATHOGENS  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 1990/04/01-2009/02/28

DESCRIPTION (provided by applicant): Control of infection with intracellular pathogens depends on the ability of the innate immune response to limit microbial replication and injury in the initial days of infection and then to efficiently facilitate the development of adaptive (antigen-specific) immunity. The innate immune system has an inherent ability to discriminate between microbial pathogens and self, a function fulfilled in part by toll-like receptors (TLRs). In addition to their important role in activating innate defenses, TLRs are thought to play an essential role in the activation of dendritic cells (DCs), which link innate and adaptive immunity, influencing the quality and magnitude of the antigen-specific immune response and the outcome of the infection. Interferon-gamma (IFN-gamma)-producing Th1 CD4+T cells and cytotoxic CD8+T cells are key components of protective antigen-specific immunity to intracellular pathogens. This proposal addresses the role of TLRs in innate immunity and in the activation of DCs leading to the induction of antigen-specific immunity to the intracellular bacterial pathogen, *Listeria monocytogenes* (Lm), and the mechanisms by which IFN-gamma is regulated in the context of this infection. Lm is a food-borne pathogen that causes severe disease in the fetus and newborn infant, and the murine model of Lm infection provides a robust system in which to address mechanisms linking innate and adaptive immunity in adults and neonates. Aim 1. Determine which TLRs contribute and the cumulative role of TLRs in the innate immune response to Lm. Aim 2. Determine the cumulative role of TLRs and MyD88 in activating DCs and in linking innate to adaptive immunity to Lm. Aim 3. Determine whether deficits in TLR/Myd88-dependent or -independent mechanisms impair the development of T cell-mediated immunity to and protection from Lm in the neonate. Aim 4. Determine the importance of conserved non-coding sequences (CSE1/2) in proper expression of IFN-gamma in the context of the immune response to Lm. These studies will provide insights into mechanisms for host defense against Lm and for the greater susceptibility of the neonate to this and related intracellular bacterial pathogens.

**Grant:** 1R01HD045783-01A1  
**Program Director:** RANKIN, TRACY L  
**Principal Investigator:** FLICKINGER, CHARLES J MD  
PHYSIOLOGY:REPRODUCTIVE  
**Title:** Antimicrobial Proteins Secreted by the Epididymis  
**Institution:** UNIVERSITY OF VIRGINIA CHARLOTTESVILLE CHARLOTTESVILLE, VA  
**Project Period:** 2004/05/15-2009/02/28

DESCRIPTION (provided by applicant): This proposal focuses on the development, regulation and discovery of naturally occurring antimicrobial proteins in the epididymis, highlighting the defensins and cathelicidins. Natural antimicrobial proteins are part of the innate immune system, and they likely protect the reproductive tract from invasion by pathogenic microbes thus helping to prevent diseases such as epididymitis. The first aim is to identify novel antimicrobial epididymal proteins in epididymal luminal fluid and to test their biological activities. The reproductive organs produce a variety of antimicrobial substances and it is likely that some of these natural antibiotics remain to be characterized. A step-wise approach will be used to test for antibacterial activity in luminal fluid from different regions of the epididymis, and subsequently to identify, characterize, and analyze the specificities of antimicrobial proteins. The second aim is to determine the pattern of expression of mRNA and protein for selected defensins and cathelicidin during development of the rat epididymis. These studies will determine the temporal onset of expression, quantify changes in expression levels during development, and localize expression to specific epithelial cell types. The third aim is to determine factors regulating expression of antimicrobial proteins in the epididymis by testing the effects of androgens, luminal fluid, exposure to bacterial products, and obstruction on expression of selected genes. Methods include detection of antimicrobial proteins by a gel overlay method and other bacteriologic assays, northern and western analyses, real time PCR, in situ hybridization, and immunohistochemistry, 2-D gel electrophoresis, microsequencing by mass spectrometry, and standard approaches of molecular biology. A set of antimicrobial proteins known to be present in the rat epididymis will be studied in aims 2 & 3: rat beta defensins 1 and 2 (RBD-1 and RBD-2), Bin1b, a cathelicidin (rCRAMP), and the defensin-like molecule E-3. The proposed studies will increase our knowledge of innate antimicrobial proteins in the male reproductive system, and they also present the opportunity for discovery and characterization of new antibiotic agents.

**Grant:** 1R03HD045725-01  
**Program Director:** KAUFMAN, STEVEN  
**Principal Investigator:** HEDGES, SPENCER R PHD  
**Title:** Bacterial vaginosis-vaginal biofilm analysis  
**Institution:** UNIVERSITY OF ALABAMA AT BIRMINGHAM BIRMINGTON, AL  
**Project Period:** 2003/12/05-2005/11/30

DESCRIPTION (provided by applicant): Bacterial vaginosis (BV) is the most prevalent cause of symptomatic vaginal discharge in the U.S. and has been associated with numerous complications including pre term delivery of infants, pelvic inflammatory disease (PID), urinary tract infections (UTI) and acquisition/transmission of sexually transmitted diseases (STDs) including human immunodeficiency virus (HIV). Control of BV has been advocated as a means of decreasing the prevalence of these complications yet the etiology of BV remains unknown and the current treatment regimens are inadequate in terms of initial cure and recurrence rates. Until further insight is gained into the infectious etiology of BV, efforts to improve therapy and to prevent complications will be difficult. Although the bacteriology of BV has been well described using standard microbiological culture methods, it has been estimated that only <1% of microorganisms are culturable using standard techniques. Denaturing gradient gel electrophoresis (DGGE) combined with PCR is a powerful molecular technique that has been used to successfully detect microbial diversity in the gastrointestinal tract and the oral cavity. We propose to use DGGE to determine differences in the microbial biofilm of women with and without BV as well as comparing women with symptomatic and asymptomatic BV to understand more about the pathogenesis of BV.

**Grant:** 1R21HD047600-01  
**Program Director:** RAJU, TONSE N.  
**Principal Investigator:** LIM, YOW-PIN PHD  
**Title:** Inter-alpha Inhibitors in Neonatal Sepsis  
**Institution:** RHODE ISLAND HOSPITAL (PROVIDENCE, RI) PROVIDENCE, RI  
**Project Period:** 2004/07/05-2006/06/30

**DESCRIPTION** (provided by applicant): The primary goal of this research is to demonstrate the feasibility of Inter-alpha inhibitor proteins (Ialp) as potential therapeutic agents in neonatal sepsis. Ialp are natural serine protease inhibitors found in relatively high concentration in human adult and newborn plasma (ranges between 0.6-1.2 mg/mL). Ialp have been implicated in play roles in inflammation, wound healing and cancer metastasis. The high level of Ialp circulating normally in plasma indicates that the proteins are essential for life and no person with complete absence of Ialp has ever been detected. A significant decrease of plasma Ialp levels occurs in plasma of adult patients with severe sepsis and septic shock and the decrease correlates with the mortality. Our preliminary results suggest a similar decrease of Ialp levels in clinically proven neonatal sepsis suggesting the involvement of Ialp in the pathogenesis of neonatal sepsis. We hypothesize that administration of Ialp restore the imbalance between these natural protective inhibitors and destructive proteases will prevent organ injury and ultimately reduce the mortality in sepsis. Our animal studies using a polymicrobial sepsis rat model of cecal ligation and puncture (CLP) have already demonstrated the beneficial effects of Ialp in maintaining hemodynamic stability, preventing organ injury, and improving survival during the progression of sepsis in adult animals.

**Grant:** 2P01HL024136-26  
**Program Director:** NOEL, PATRICIA  
**Principal Investigator:** CAUGHEY, GEORGE H. MD  
**Title:** Mechanisms of Remodeling in Chronic Airway Inflammation  
**Institution:** UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA  
**Project Period:** 1997/07/01-2009/06/30

DESCRIPTION (provided by applicant): This application requests funding to continue multidisciplinary studies of mechanisms of airway remodeling in chronic inflammation. Drs. Basbaum, Caughey, Killeen, and McDonald have collaborated closely in this Program Project Grant for many years and will continue their efforts to understand the cellular and molecular pathophysiology of airway remodeling. They will focus on how changes in epithelial and inflammatory cells, blood vessels, lymphatics, and proteases contribute to airway remodeling. Using state-of-the-art molecular, cellular, morphological, immunological, and genetic approaches, the multidisciplinary team will approach the problem in four ways. Project 1, led by Dr. Basbaum, will explore mechanisms of pathological down-growth of surface epithelium to form submucosal glands, focusing on the roles of lymphocytes and the metalloproteinase inducer EMMPRIN in gland bud formation. Project 2, led by Dr. Caughey, will investigate roles of extracellular proteases from airway epithelial cells, mast cells, and inflammatory cells in remodeling of the airway microvasculature and epithelium, emphasizing in vitro explorations of the molecular behavior and targets of proteases and in vivo studies of selected proteases and their targets in genetically engineered mice. Project 3, led by Dr. Killeen, will examine molecular mechanisms whereby T and B lymphocytes cooperate in triggering antibody-mediated remodeling of the airway microvasculature and epithelium and will identify the mediators involved. Project 4, led by Dr. McDonald, will examine the mechanisms, consequences, and reversibility of angiogenesis and lymphangiogenesis to explore the gate-keeper function of the microvasculature in sustaining chronic airway inflammation and the role of defects in lymphatic growth in mucosal edema. Novel transgenic mouse models and mice with chronic airway inflammation from *Mycoplasma pulmonis* infection will be used in many investigations. To facilitate these studies, each of the projects will be supported by a Mouse Genotyping and Infection Core, led by Dr. Killeen. The Program Project team has a long tradition of collaborative research and use of multidisciplinary strategies for studying airway inflammation and remodeling. Collectively, their powerful armory of experience and skills in cellular and molecular biology, enzymology, microscopic imaging, and immunology will be used with innovative biochemical, in vivo model systems to solve the mysteries of airway remodeling in chronic disease. Understanding the causes of remodeling will suggest new strategies to prevent or reverse the long lasting changes in the airway wall typical of asthma, bronchitis, cystic fibrosis, and other chronic inflammatory airway diseases affecting a growing share of population.

**Grant:** 1P01HL073750-01A1

**Program Director:** LINK, REBECCA

**Principal Investigator:** CASTELLINO, FRANCIS J  
PHD  
BIOCHEMISTRY:BIOCHEMISTR  
Y-UNSPEC

**Title:** Pathophysiologies Involving Hemostasis-related Genes

**Institution:** UNIVERSITY OF NOTRE DAME  
NOTRE DAME, IN

**Project Period:** 2004/05/01-2009/04/30

It is becoming increasingly clear that genes traditionally associated with hemostasis function in many other diverse pathophysiologic processes. Examples are the involvement of gene products related to fibrinolysis, e.g., plasminogen, plasminogen activators, and plasminogen activator inhibitors, with their roles in cancer, wound healing, and angiogenesis. These same genes, as well as products of genes of relevance to coagulation, e.g., Tissue Factor, and anticoagulation, e.g., Protein C, also function in embryogenesis, cancer, and acute and chronic inflammatory-based processes, among others. Thus, hemostasis-related genes serve as links between different pathways in health and disease. This Program Project Grant (PPG) builds on existing strengths and integrates the research efforts of experienced investigators who have made major contributions to our understanding of the protein chemistry, molecular and cell biology, gene targeting, and pathophysiologies of proteins and genes associated with hemostasis. The focus of this PPG is the definition of the in vivo roles of hemostasis-related genes in: the relationships between disseminated intravascular coagulation, systemic inflammation, and organ damage during the progression of sepsis (Project by Castellino); tumorigenesis, metastasis, and angiogenesis (Project by Ploplis); and embryonic and perinatal survival of offspring, as well as in vivo thrombus formation (Project by Rosen). Three core units are proposed as necessary to centrally support this group of projects: (1) an Administrative Core, (2) an Anatomic Pathology Core, and (3) a Mouse Breeding and Husbandry Core. The Project and Core Leaders have a long history of productive interactions with each other and are all based in an infrastructure-rich center devoted to in vivo and in vitro studies of coagulation, anticoagulation, and fibrinolysis. The projects proposed will utilize the same administrative, histopathology, and mouse cores. The PPG will allow increased interactions and collaborations to occur between the laboratories of the Project Leaders in studying the functional roles of hemostasis-related genes, and the overall program that results from these combined efforts will exceed the sum of the individual parts. The research efforts and productivity of students and postdoctorals will benefit greatly from the interactions of the individual laboratories and cores that will result from the PPG, and will serve as a resource for a continual flow of independent investigations in these research areas.

**Grant:** 1P01HL073907-01A1  
**Program Director:** NOEL, PATRICIA  
**Principal Investigator:** MARTIN, RICHARD J MD OTHER AREAS  
**Title:** The Effect of Mycoplasma on Chronic Asthma  
**Institution:** NATIONAL JEWISH MEDICAL & RES CTR DENVER, CO  
**Project Period:** 2004/07/05-2009/03/31

DESCRIPTION (provided by applicant): This Program Project Grant (PPG) will bring together 16 investigators in four Projects and two Cores to continue our work in the area of the link between chronic infection (*M. pneumoniae*) and chronic asthma. There have been numerous recent publications suggesting that in a subgroup of asthmatics, approximately 50% have an associated atypical bacterial process. Thus, this PPG will focus on the *M. pneumoniae*-asthma interaction. Project 1 will evaluate the effect of *M. pneumoniae* on neurogenic inflammation inducing fibroblast proliferation and altering airway structure (remodeling) and function. This will be done using asthmatic airway epithelial cells and tissue. The focus of Project 2 is to examine the interactions of surfactant proteins (SP)-A and -D, the first line airway defense mechanisms and *M. pneumoniae*. This project will also identify the domains of the protein that are required for the amplification of the inflammatory response. In addition to this in vitro work, in vivo mouse work will determine how the mycoplasma response is modulated by SP-A and SP-D. Project 3 will use a mouse model of a mycoplasma induced chronic "asthma-like" state by the interaction of allergen and infection exposures. This model will be used to understand the development of chronic bronchial hyper-responsiveness and airway remodeling. Project 4 will use an epithelial human cell line to investigate the effects of *M. pneumoniae* on mast cell function. Each project is complementary and interactive, thus the critical mass of scientists will be able to work at a higher and interactive level through the PPG. The Administrative Core will function as the fulcrum for the projects and the Histopathology and Microbiology Core will serve each project. The goal of understanding the interaction between *M. pneumoniae* and asthma will be obtained through this PPG and will ultimately give new knowledge to the understanding of asthma pathophysiology and potential future novel therapies.



**Grant:** 1P01HL076100-01  
**Program Director:** PEAVY, HANNAH H  
**Principal Investigator:** SHELLITO, JUDD E MD OTHER AREAS  
**Title:** Host Defense Against HIV-related Pulmonary Infections  
**Institution:** LOUISIANA STATE UNIV HSC NEW ORLEANS NEW ORLEANS, LA  
**Project Period:** 2004/02/10-2009/01/31

DESCRIPTION (provided by applicant): Pulmonary infections are frequent complications of HIV infection and a common cause of death in patients with AIDS. Pulmonary pathogens complicating HIV infection include bacteria (pneumonia), fungi (*Pneumocystis carinii*), and mycobacteria (tuberculosis). Antimicrobial therapy is possible for many of these infections, but such therapies may not be available in developing countries and antibiotic resistance is an increasing problem. A more direct solution to the problem of HIV-related pulmonary infections is to correct the defects in host defense caused by HIV infection. The long-term objective of this Project is to identify new methods to augment host defense against pulmonary infection in HIV-infected patients. In this application, we will direct and coordinate a group of established investigators in host defense to conduct research targeted to HIV-related pulmonary infections. The Project theme is to explore genetic approaches to enhance the delivery of immune effector cells into lung tissue in order to augment clearance of HIV-related pulmonary infections. We postulate that genetic approaches can be used to either increase local signals for recruitment of immune effector cells into infected lung tissue or to increase the pool of circulating effector cells available for recruitment. Four interactive research projects will address: 1. CXCR3 ligands as signals for lymphocyte recruitment in host defense against *P. carinii* (Dr. Shellito) 2. CD4-independent vaccine strategies for pulmonary infections (Dr. Kolls) 3. Prime/boost vaccination strategies for pulmonary tuberculosis (Drs. Ramsay, Mason) 4. Strategies to increase hematopoietic responses to bacterial pneumonia (Drs. Schwarzenberger, Nelson, and Bagby) The research projects will be supported by 3 core components: Administrative Core (Dr. Shellito), Immunology Core (Dr. Zhang), Vector Core (Dr. Reiser) Project research will develop and validate new methods of immune augmentation in animal models of pulmonary infection relevant to HIV infection. Data generated will then provide a foundation for future applications to HIV-infected patients.

**Grant:** 2R01HL031237-20  
**Program Director:** HARABIN, ANDREA L.  
**Principal Investigator:** KUNKEL, STEVEN L. PHD PARASITOLOGY, OTHER  
**Title:** Monokine Gene Expression/Regulation in Lung Injury  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 1984/01/01-2008/02/28

**DESCRIPTION** (provided by applicant): The early events of severe sepsis and acute lung injury set in motion a cascade of mechanisms which significantly contribute to the morbidity and mortality observed during the first few days of this syndrome. While sepsis has often been viewed as a deadly acute disease, survivors also suffer long-term consequences. Clinical data underscores the enigma of subsequent high mortality rates associated with patients who are long-term survivors of the acute septic episode. For example, within one year of surviving severe sepsis, there is a 26% predicted mortality rate, with many patients succumbing to lung complications. In this renewal application we will expand upon our previous investigations and focus on the cellular and molecular mechanisms which dictate the longer-term sequela of sepsis and related lung injury. We have established a model of experimental sepsis (cecal ligation and puncture-CLP) which results in an approximate 60% survival rate. Our preliminary studies have demonstrated that these survivors are susceptible to either a fungal or bacterial infection with 100% mortality when challenged 2-3 weeks post recovery from the initial CLP; with changes in cytokine and toll-like receptor (TLR) expression and alterations in lung leukocyte populations. No challenged sham control animals died. We hypothesize that the lung becomes uniquely predisposed to infection for extended periods of time after animals survive severe sepsis via mechanisms that include alterations in the inflammatory cytokines IL-12 and IL13, an increase in immunoregulatory chemokines MCP-1 and C10, and alterations in innate immunity. Our studies will focus on the following Specific Aims: To compare and contrast mediators, TLR, and lung immune cells in models of long-term survivors of mild and severe sepsis, as these animals respond to a subsequent challenge. To investigate the mechanisms by which IL-12 and IL-13 contribute to innate immunity after pathogen challenge of surviving septic animals by influencing cytokine expression profiles, TLR expression, and lung leukocyte elicitation and activation. To assess the mechanistic contribution of CC chemokine with unique immunoregulatory activity to the long term modulation of the lung post severe sepsis. To determine the contribution of lung resident, structural cells as they participate in the innate response of long-term sepsis survivors to a pathogen challenge. Our long term goals are to demonstrate the mechanistic contributions of cytokines, TLR, and dendritic cells to the long term problems of severe sepsis and acute lung injury.

**Grant:** 2R01HL055934-10  
**Program Director:** PEAVY, HANNAH H  
**Principal Investigator:** LIMPER, ANDREW H  
**Title:** Control of the Pneumocystis carinii Life Cycle  
**Institution:** MAYO CLINIC COLL OF MEDICINE, ROCHESTER, MN  
ROCHESTER  
**Project Period:** 1995/09/30-2009/08/31

DESCRIPTION (provided by applicant): Pneumocystis pneumonia remains a significant cause of mortality and morbidity in immunocompromised patients. Our studies have demonstrated that binding of Pneumocystis to alveolar epithelial cells is a central feature of infection, leading to organism proliferation. Pneumocystis attachment to epithelial cells is mediated through host extracellular matrix proteins, particularly fibronectin and vitronectin. We recently demonstrated that Pneumocystis expresses an INT1 gene, homologous to integrin-like molecules, which promotes organism adherence to matrix-coated surfaces. Our investigations further reveal that binding of Pneumocystis to lung cells or matrix specifically induces expression of particular signaling kinases in the organism, most notably STE20 and CBK1. Interestingly, both of these kinases have critical activities in regulating beta-glucan cell wall assembly and stimulating proliferation of fungi. We, therefore, hypothesize that binding of Pneumocystis to lung extracellular matrix proteins and epithelial cells, activates specific kinase signaling cascades that stimulate life cycle progression, promoting cell wall assembly and proliferation of the organism. These concepts will be addressed through four independent but interrelated specific aims. In Aim 1, we will define the role of Pneumocystis INT-1 in mediating adherence of the organism to lung epithelial cells. Under Aim 2, we will assess the mechanisms by which Pneumocystis adherence to matrix and lung cells promotes activation of the upstream Pneumocystis STE20 kinase, and will determine the interactions of STE20 with the downstream CBK (Cell Wall Biosynthesis Kinase) of the organism. Next, Aim 3 will evaluate whether Pneumocystis adherence will promote expression and activity of the beta-glucan cell wall assembly machinery in the organism, with subsequent transition of trophic forms to cysts, a critical component of the organism's life cycle. Finally in Aim 4, we will utilize cell wall assembly inhibitors, which are active in Pneumocystis, to further dissect life cycle progression and infection in immune suppressed rodents inoculated with purified Pneumocystis trophic forms. We will determine the effect of cell wall assembly inhibition on respective trophic form and cyst populations associated with (-glucan cell wall generation in these animals. Specific interruption of Pneumocystis adherence to lung epithelial cells and suppression of encystment, with reduced organism proliferation, may yield novel therapeutic approaches for the prevention and management of Pneumocystis pneumonia.

**Grant:** 2R01HL056036-08  
**Program Director:** TOLUNAY, ESER  
**Principal Investigator:** KUO, CHO-CHOU  
**Title:** Role of Chlamydia Pneumoniae in Atherosclerosis  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 1997/04/01-2008/03/31

DESCRIPTION (provided by applicant): Chlamydia pneumoniae is a common human respiratory pathogen. In recent years the scope of C. pneumoniae in human diseases has been extended to the cardiovascular diseases. The studies on seroepidemiology, detection of the organism, and animal models have provided a strong evidence indicating that C. pneumoniae and hyperlipidemia are co-risk factor of atherosclerosis. Because coronary heart disease is a leading cause of death in this country and worldwide, the overall goal of this proposal is to investigate the immunopathogenic mechanisms by which C. pneumoniae promotes the development of vascular disease and to develop the secondary preventive measures for C. pneumoniae-associated atherosclerosis. This research proposal will exploit the recent findings from our studies on the mouse models of C. pneumoniae and atherosclerosis and cell cultures on C. pneumoniae infection using arterial wall cells. The specific aims are to 1) test the hypothesis that the tropism of C. pneumoniae is dependent on previous activation of the endothelium and expression of adhesion molecules, which facilitates C. pneumoniae homing to and establishing persistent infection at the lesion site to accelerate atherosclerosis; 2) analyze gene expression in foam cell macrophages induced by C. pneumoniae infection by the microarray technique; 3) determine whether infection of hyperlipidemic mice with C. pneumoniae increases expression of Egr-1 responsive genes in the artery wall and whether infection accelerates development of atherosclerotic lesions in Egr-1 knockout mice; and 4) evaluate the ligand-receptor based therapy for prevention of C. pneumoniae-associated atherosclerosis in mice by administering food or water supplemented with mannose 6-phosphate/mannan and retinoic acid. The proposed studies should contribute to the understanding of the disease process and development of better eradication or preventive measures for C. pneumoniae infection and reduction of atherosclerosis and coronary heart disease.

**Grant:** 2R01HL057879-06A1  
**Program Director:** PEAVY, HANNAH H  
**Principal Investigator:** WEIDEN, MICHAEL D MD  
**Title:** HIV/TB Interaction in the Lung  
**Institution:** NEW YORK UNIVERSITY SCHOOL OF MEDICINE NEW YORK, NY  
**Project Period:** 1999/04/01-2009/07/31

DESCRIPTION (provided by applicant): HIV/TB interaction in the lung has focused on regulation of HIV-1 replication in alveolar macrophages (AM), the major source of viral replication in tuberculosis (TB). Work performed in this laboratory has demonstrated: 1) Production of inhibitory C/EBPbeta is an interferon (IFN) effect. It is also induced by other innate immune mediators such as SP-a. Once expressed, inhibitory C/EBPbeta suppresses HIV-1 replication and most proinflammatory cytokine promoters in resting AM. 2) As monocytes differentiate to macrophages, they gain the ability to produce a dominant negative C/EBPbeta transcription factor. 3) During TB, contact between lymphocytes and AM drives high-level HIV-1 replication in AM. Both lymphocyte/AM contact and cytokines are required for maximal LTR activation. Lymphocyte/AM contact down-regulates the dominant negative C/EBPbeta, de-repressing the HIV-1 LTR; while cytokines activate NF-kappaB, stimulating the HIV-1 LTR. 4) In mice, CD40 expression is required for de-repression during sepsis. Preliminary data now demonstrate that PU.1 and CREM are expressed in resting AM but not during TB. Both PU.1 and CREM are transcriptional repressors in other systems. In addition, a subset of AIDS patients with TB demonstrates neutrophil (PMN) predominant inflammation. PMN stimulate HIV-1 replication and mutation in vivo and in vitro. Full induction of HIV-1 replication and LTR function requires PMN contact and soluble factors. Like lymphocytes, PMN express CD40L and CD28. Unlike lymphocytes, PMN express LFA-1, which binds macrophage ICAM-1, and PMN-derived peroxide is a soluble factor that activates NF-kappaB. PMN contact down-regulates inhibitory C/EBPbeta, CREM and PU.1 in AM. TB patients have elevated levels of soluble ICAM-1, which recruits CD40L to PMN lipid rafts. Antibodies to CD40L, CD28 and CD11a inhibit the activity of PMN lipid rafts. PMN lipid rafts and cross-linking antibodies to CD40, B7 and ICAM-1 aggregate macrophage CD40, B7 and ICAM-1 and abolish inhibitory C/EBPbeta expression. These data led to the hypothesis that inhibitory C/EBPbeta is one of multiple repressors inhibiting HIV LTR activity in resting AM. Further, PMN are a cellular component of the innate immune response that can de-repress the LTR by cellular contact and activate the LTR by soluble factors. This two-step process contributes to high-level HIV-1 replication in AM during opportunistic infection. This proposal will investigate the role of PU.1 and CREM as inhibitors of HIV-1 replication in AM and the role of PMN in enhancing HIV-1 replication in the lung.

**Grant:** 2R01HL058795-06A1  
**Program Director:** DENHOLM, ELIZABETH M  
**Principal Investigator:** KORFHAGEN, THOMAS R MD  
**Title:** Sufactant Protein-A and Lung Defense  
**Institution:** CHILDREN'S HOSPITAL MED CTR CINCINNATI, OH  
(CINCINNATI)  
**Project Period:** 1998/04/01-2009/01/31

DESCRIPTION (provided by applicant): Pulmonary inflammatory processes due to bacterial pneumonia impose a considerable clinical burden of morbidity and mortality in the US and other countries. A number of microbes considered as potential bioterrorist threats cause severe pulmonary inflammation. During the previous funding period, studies using transgenic mice, demonstrated that surfactant protein-A (SP-A) reduces inflammation caused by microbes and microbial products. Studies from patients with pneumonia or cystic fibrosis (CF) demonstrated reduced concentrations of SP-A suggesting that SP-A modulates the extent of microbial induced pulmonary inflammation. The goal of the present application is to determine mechanisms whereby SP-A regulates pulmonary inflammatory responses. Recent studies have demonstrated important roles for toll-like receptors (TLR) in inducing inflammatory responses. TLR4 is a major receptor for LPS and gram-negative bacteria. LPS binds to CD14 and LPS/CD14 interacts with MD-2/TLR4 forming a cell surface tripartite receptor complex that transduces intracellular signals leading to activation of cytokines and other inflammatory modulators. SP-A does not bind smooth forms of LPS but SP-A blocks smooth LPS induced cytokine production in vivo and in vitro. The lack of binding to smooth LPS suggests that SP-A cannot simply be sequestering LPS from interactions with the TLR complex. TLR4, CD14, and MD-2 RNA are present in alveolar macrophages and mouse lung epithelial cells supporting the central hypothesis that SP-A alters inflammatory responses in the lung by reducing smooth LPS signaling through TLR-4 components. This hypothesis will be tested using smooth LPS mediated induction of NF-kappaB in cell transfections or LPS and gram-negative infection in mouse models to complete the following aims: (1) The SP-A structures and LPS receptor components that functionally interact to cause SP-A inhibition of LPS mediated signaling will be identified in vitro; (2) Mechanisms by which SP-A inhibits LPS mediated signaling will be determined by testing if SP-A alters interactions between TLR4 components necessary for LPS signaling in vitro; and (3) Structural domains of SP-A required for SP-A inhibition of LPS or gram-negative bacterial mediated signaling in vivo will be identified. The present application seeks to identify novel mechanisms of SP-A regulation of pulmonary inflammatory responses with the goal of identifying novel approaches to reducing pulmonary inflammation.

**Grant:** 2R01HL058897-08  
**Program Director:** REYNOLDS, HERBERT Y  
**Principal Investigator:** PETERS-GOLDEN, MARC L.  
**Title:** Eicosanoids and Lung Macrophage Antimicrobial Mechanisms  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 1997/07/10-2008/06/30

DESCRIPTION (provided by applicant): Modulating innate pulmonary defense mechanisms for therapeutic advantage requires a better understanding of the molecules that mediate the antimicrobial actions of phagocytic cells. Although most research in this area has focused on peptide mediators, our laboratory has established an important role for eicosanoid lipid mediators derived from arachidonic acid. Our work has demonstrated that leukotrienes (LTs) B4 and D4 enhance, while prostaglandin E2 (PGE2) inhibits, alveolar macrophage (AM) phagocytosis of immunoglobulin G (IgG)-opsonized microbes, a process that proceeds via Fcγ receptor (FcR)-mediated binding and signaling. Moreover, decreases in the ratio of endogenously generated LTs:PGE2 may contribute to the increased susceptibility to infection observed in such conditions as HIV infection, malnutrition, and aging. Little is known about signal transduction in primary AMs in response to ligation of either FcR or G protein-coupled eicosanoid receptors. This proposal focuses on how LTs and PGE2 modulate the cascade of signal transduction events downstream from FcR which includes Syk, phosphoinositide 3-kinase, Akt, focal adhesion kinase, and extracellular signal-related kinase. The hypothesis is that the modulatory effects of eicosanoids on AM phagocytosis reflect the amplification or suppression of these signals by LTs and PGE2, respectively, which occur in part via alteration in intracellular cyclic adenosine monophosphate. The hypothesis will be tested in a series of in vitro experiments utilizing cultured AMs challenged with IgG-coated erythrocytes. The receptors, G proteins, and signaling targets through which exogenous and endogenous eicosanoids act to modulate phagocytosis will be defined by employing a combination of genetic and pharmacologic tools. By providing new insight into FcR signaling in AMs and the mechanisms by which eicosanoids modulate this process, these studies will enhance our knowledge of an understudied but clinically relevant arm of pulmonary innate immunity.

**Grant:** 2R01HL064560-07  
**Program Director:** COLOBINI-HATCH, SANDRA  
**Principal Investigator:** CHEN, ZHENG W MD BIOMEDICAL RESEARCH  
**Title:** Macaque Models of AIDS-Related Tuberculosis and T Cell  
**Institution:** UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL  
**Project Period:** 1999/09/30-2009/08/31

DESCRIPTION (provided by applicant): Tuberculosis remains one of the leading public health problems in the world today. HIV epidemic clearly is the most important risk factor. While human CD4 T cells play a crucial role in immune protection against *M. tuberculosis* infection, other T cell populations are not well characterized for their roles in immunity to tuberculosis. Phosphoantigen-specific V $\alpha$ 2V $\beta$ 2 T cells exist only in primates and constitute 60-95% of total human  $\gamma\delta$  T cell population in the blood. We have recently demonstrated that macaque V $\alpha$ 2V $\beta$ 2 T cells can mount adaptive immune responses during BCG and *M. tuberculosis* infections, and that the capacity of memory V $\alpha$ 2V $\beta$ 2 T cells to rapidly expand coincides with immunity to acutely fatal tuberculosis. We therefore hypothesize that V $\alpha$ 2V $\beta$ 2 T cells play a role in immunity to tuberculosis and AIDS-related reactivation tuberculosis. To test this hypothesis, we have adapted macaque models of pulmonary tuberculosis and AIDS-related tuberculosis-like disease. For this project, we will I. Determine if enhanced activation of V $\alpha$ 2V $\beta$ 2 T cells by phosphoantigen treatment during *M. tuberculosis* infection can attenuate disease course of tuberculosis in immune competent and SIV mac-infected macaques. II. Determine if restored V $\alpha$ 2V $\beta$ 2 T cell responses during antiretroviral therapy or combined antiretroviral-phosphoantigen treatment contribute to protection against SIV-related tuberculosis-like disease or SHIV-induced reactivation tuberculosis. III. Determine the utility of vaccination of V $\alpha$ 2V $\beta$ 2 T cells in delay or prevention of tuberculosis in immune competent and SHIV-infected monkeys.



**Grant:** 2R01HL065397-05  
**Program Director:** BERBERICH, MARY ANNE  
**Principal Investigator:** JOBE, ALAN H MD OTHER AREAS  
**Title:** New Mediators of Clinical Lung Maturation  
**Institution:** CHILDREN'S HOSPITAL MED CTR CINCINNATI, OH  
(CINCINNATI)  
**Project Period:** 2000/09/01-2008/08/31

DESCRIPTION (provided by applicant): Clinical lung maturation is frequent in very low birth weight preterm infants and early lung maturation is associated clinically with the chronic chorioamnionitis that seems to cause preterm delivery. Intra-amniotic endotoxin causes chorioamnionitis, lung inflammation and striking lung maturation in fetal sheep. Intra-amniotic IL-1 also causes lung maturation, and both IL-1 and endotoxin induce the lung maturation response by direct contact with the fetal lung and not by inducing a systemic response. Other inflammatory mediators such as TNFalpha and IFNgamma have no effect on the fetal lung. Preliminary data suggest that the fetal airways produce mediators that recruit (IL-8) W that recruit WBC to the fetal lungs and that those cells then amplify the maturation signal, which may be IL-1beta, resulting in increased surfactant and lung structural changes. This proposal explores the pathways leading from the intra-amniotic pro-inflammatory mediator to lung maturation using the fetal sheep model. Experiments will test if IL-1 is the proximal mediator of lung maturation, if IL-8 produced by the airway recruits WBC to the fetal lungs, if those cells are required for lung maturation, and if these pathways are NFKappaB dependent. The innate host response to inflammation is mediated by receptors for pathogen component recognition domains, the Toll-Like Receptors (TLRs). There is no information about TLR function in the fetus. Because chorioamnionitis is caused by different organisms, we will characterize the fetal lung responses to intra-amniotic TLR agonists for TLR2, TLR3, TLR4, TLR5 and TLR9. These data together with identification of the signaling pathways for endotoxin (TLR4)/IL-1 will elucidate the links between antenatal infection and lung maturation. A goal is to identify a mediator with potential for clinical use to induce lung maturation.

**Grant:** 1R01HL069431-01A2  
**Program Director:** REYNOLDS, HERBERT Y  
**Principal Investigator:** SIMECKA, JERRY W PHD MICROBIOLOGY, OTHER  
**Title:** Dendritic cells & immunity in mycoplasma pneumonia  
**Institution:** UNIVERSITY OF NORTH TEXAS HLTH SCI CTR FORT WORTH, TX  
**Project Period:** 2004/02/15-2008/01/31

DESCRIPTION (provided by applicant): The broad, long-term objectives of this project are to develop approaches to prevent and treat respiratory diseases. We found that different T cells populations modulate inflammatory responses of murine mycoplasma pneumonia, and the factors that determine the type of T cell responses need to be understood. The role of dendritic cells (DCs) and other antigen presenting cells, e.g. macrophages, in Mycoplasma disease is unknown, but because T cell responses in mycoplasma disease play such a critical role, DCs and macrophages likely impact on disease pathogenesis and influence the generation of protective immunity. We hypothesize that manipulating DC can predictably change the types and intensity of immune reactions in the lung against mycoplasma infection, and this will impact on the severity and resistance to disease. In addition, pulmonary macrophages may also impact on mycoplasma immunity, possibly through support of Th1-type responses. The impact of pulmonary DCs and macrophages activity needs to be compared. In this proposal, we will address the following questions: 1) Do changes in pulmonary DCs and macrophages from naive or mycoplasma-infected mice influence the ability to activate and modulate T cell differentiation and activation? 2) Can mycoplasma-antigen pulsed DCs or macrophages generate protective or immunopathologic responses against mycoplasma? 3) Can altering DC function with regulatory cytokines influence pulmonary immune responses and mycoplasma respiratory disease? 4) Do the DCbeta-chemokines, ABCD-1 and ABCD-2 (TARC), play a role in pulmonary immunity against mycoplasma? The experimental design is as follows: 1) DCs and macrophages from the respiratory tracts of mycoplasma-infected mice will be evaluated for their ability activate in vitro and in vivo T cell responses against an unrelated antigen (OVA). 2) DCs and macrophages will be pulsed with mycoplasma antigen and their ability to generate resistance or immunopathology after intratracheal inoculation and subsequent challenge with mycoplasma will be determined. 3) DC will be treated in vitro with regulatory cytokines, IFNgamma, IL-12, IL-4 TGF-beta or IL-10, and pulsed with mycoplasma antigen, and their ability to generate resistance or immunopathology against mycoplasma will be determined by their intratracheal inoculation; and 4) T cells from lungs of Mycoplasma infected mice will be examined for expression of receptors for ABCD-1 and ABCD-2 and their ability to respond to these chemokines. Mice will be treated with neutralizing monoclonal antibodies to determine the role of ABCD-1 and ABCD-2 in protective immunity and immunopathologic responses against mycoplasma.

**Grant:** 1R01HL069809-01A2

**Program Director:** HARABIN, ANDREA L.

**Principal Investigator:** WIENER-KRONISH, JEANINE P MD  
MULTIDISCIPLINARY: MULTIDISCIPLINARY, BASIC MED

**Title:** Gene Expression and Pathogenicity of *P. aeruginosa*

**Institution:** UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA

**Project Period:** 2003/12/11-2007/11/30

**DESCRIPTION** (provided by applicant): We are proposing a translational investigation, including clinical as well as basic scientists, to test the hypothesis that specific *Pseudomonas* virulence products determine whether lung infection occurs. The results from the proposed experiments will be used to develop diagnostic tools that can identify virulent *Pseudomonas* in patients. The results will also identify critical bacterial gene products involved in lung injury. The present application carefully dissects associations between bacterial virulence and lung injury so that future mechanistic investigations in patients can be proposed. Without the data from the proposed investigations, we do not have the information to proceed. We already have *Pseudomonas* strains obtained from a cohort of intubated, mechanically ventilated patients from another grant. Our first aim is to perform a genomic analysis of *Pseudomonas* strains obtained from 2 distinct patient populations; intubated, ventilated patients with signs of lung infections that have disseminated and intubated, ventilated patients who have no sign of disease. Using microarray analysis, we will determine the significantly different genes between the *Pseudomonas* strains found in these two patient groups and produce consensus primers to these genes. We will then utilize these consensus primers in a prospective investigation of 300 intubated, ventilated patients to confirm the prevalence of the genetic differences in colonized vs infected intubated, ventilated patients. The second aim is to compare the *in vivo* virulence gene expression of *Pseudomonas* strains obtained from colonized patients to the expression profile of the strains obtained from patients who have *Pseudomonas* lung infections. Analysis of bacterial gene expression *in vivo* will be done using real-time PCR. The results of all these experiments will allow us to prove (or disprove) that there are different genes or different patterns of toxin gene expression between the *Pseudomonas* strains infecting patients and those colonizing patients. In the final aim, we will evaluate the interaction of type III toxin genes *in vivo*. By utilizing an isogenic bacterial strain (PA103) that has each of the toxin genes added alone or in combination, we will be able to define the effects of each of the type III toxins as well as other genes we find of significance in the first two aims.

**Grant:** 1R01HL070891-01A1  
**Program Director:** HARABIN, ANDREA L.  
**Principal Investigator:** STECENKO, ARLENE A MD PEDIATRICS:PULMONARY DISEASE  
**Title:** C/EBP Beta Regulation of Lung Inflammation  
**Institution:** EMORY UNIVERSITY ATLANTA, GA  
**Project Period:** 2004/05/12-2009/04/30

**DESCRIPTION** (provided by applicant): Ordinarily, neutrophilic inflammation is an acute response that either resolves or transitions to a chronic lymphocytic process. However, in several lung diseases including sepsis induced acute lung injury, neutrophil-dominated inflammation persists throughout the course of the disease. Activation of the transcription factor NF- $\kappa$ B is a proximal trigger for neutrophilic inflammation, but factors responsible for termination of the neutrophilic response (or failure to do so) and initiations of the repair process are less clear. In normal human airway epithelial cells and in preliminary experiments in animals, we have evidence that termination of the inflammatory response in the lungs depends on selective increased production of the inhibitory isoform of the transcription factor C/EBP $\beta$  (called p20). The DNA binding sites for C/EBP $\beta$  and NF- $\kappa$ B are in close proximity in the promoter region of several genes and interactions between the two factors results in synergistic enhancement of gene expression by the activator C/EBP $\beta$  isoform and inhibition by the dominant negative inhibitory isoform (p20). As both experimental tools and potential therapeutics, we have developed two unique recombinant fusion proteins rendered cell permeable by inclusion of a membrane translocation sequence (MTS). One of these proteins (I $\kappa$ B((N)-MTS) inhibits activation of NF- $\kappa$ B and the other (p20-MTS) delivers the inhibitor isoform of C/EBP $\beta$  to cell nuclei. Our goal is to use these tools to elucidate the roles of the two transcription factors, NF- $\kappa$ B and C/EBP $\beta$ , in the pathogenesis of endotoxin induced lung injury and the early phase of lung repair in a well established sheep model. Specifically, we aim to: 1) in anesthetized sheep, determine the early time course of NF- $\kappa$ B and C/EBP $\beta$  activity in liver and lung following endotoxemia, relate these changes to measurements of lung function and cellular and biochemical responses, and determine the effects of intravenous delivery of either the inhibitor of NF- $\kappa$ B or of C/EBP $\beta$  on the response to endotoxemia; 2) in chronically instrumented, unanesthetized sheep, determine effects of intravenous administration of either the inhibitor of NF- $\kappa$ B or the inhibitor of C/EBP $\beta$  on sub-acute physiologic, cellular and biochemical responses to endotoxin and on the early phase of recovery of the lungs from endotoxin-induced injury; 3) in chronically instrumented, unanesthetized sheep, determine effects of aerosol administration of either inhibitor on the response to endotoxin and on the early phase of recovery of the lungs from injury; and 4) from the above studies, select the most promising therapeutic regimen and determine effects of its administration four hours after endotoxemia on the subsequent course of the endotoxin response in chronically instrumented unanesthetized sheep.

**Grant:** 1R01HL071091-01A2  
**Program Director:** LIANG, ISABELLA  
**Principal Investigator:** GOTTLIEB, ROBERTA A MD MEDICINE  
**Title:** Cytochrome P450 in Reperfusion Injury  
**Institution:** SCRIPPS RESEARCH INSTITUTE LA JOLLA, CA  
**Project Period:** 2003/12/01-2007/11/30

**DESCRIPTION** (provided by applicant): In this proposal, we present new data implicating cytochrome P450 monooxygenases (CYP) in myocardial ischemia/reperfusion (I/R) injury. We have shown that inhibition of CYP with compounds such as chloramphenicol or sulfaphenazole can reduce tissue injury following myocardial ischemia and reperfusion. Importantly, infarct size is reduced by up to 70 percent by pretreatment with chloramphenicol or sulfaphenazole. In the isolated perfused heart model, these drugs are beneficial even when administered after ischemia. In Aim 1 we will extend the existing preliminary studies with the following objectives: to establish the minimum effective dose in the isolated perfused heart model; to establish how long a delay before drug administration is still protective; and to assess efficacy of sulfaphenazole (the most potent lead compound identified thus far) in a model of regional ischemia and reperfusion in the anesthetized rabbit. Since several of the CYP inhibitors we have shown to be cardioprotective are already safely used in humans for other purposes, it is hoped that these preclinical studies will lay the groundwork for a subsequent clinical trial. We will also establish whether upregulation of CYPs in the heart increases I/R injury. In Aim 2, we seek to understand the mechanism of cardioprotection by these CYP inhibitors, as this will yield insights into the role of CYPs in causing myocardial damage after ischemia/reperfusion. To this end, we will identify the CYP isoforms in heart that are inhibited by sulfaphenazole and chloramphenicol, and will consider the two likely mechanisms of injury-production of excessive reactive oxygen species, and the dysregulated metabolism of arachidonic acid to vasoactive and cardiotoxic eicosanoids. In Aim 3 we consider the potential effect of CYPs on the SR Ca<sup>2+</sup> ATPase and the mitochondrial KATP channel. We address the potential role of CYPs in apoptosis, based on the finding that the anti-apoptotic proteins ARC (apoptosis repressor with CARD domain) and Bcl-xL inhibit CYP activity, while pro-apoptotic tBid stimulates CYP activity in rat liver microsomes. This proposal identifies cytochrome P450 monooxygenases as a previously underestimated factor in myocardial reperfusion injury, and establishes the basis for a novel therapeutic approach

**Grant:** 1R01HL072817-01A1  
**Program Director:** PEAVY, HANNAH H  
**Principal Investigator:** GERALD, LYNN B MS  
**Title:** Effectiveness of the TB Contact Priority Model  
**Institution:** UNIVERSITY OF ALABAMA AT BIRMINGHAM BIRMINGHAM, AL  
**Project Period:** 2004/05/31-2009/03/31

**DESCRIPTION** (provided by applicant): Investigation of contacts of patients with active pulmonary tuberculosis (TB) is an important epidemiological tool in TB control. Unfortunately, due to budgetary and resource constraints, many health departments struggle to fulfill their responsibility of TB control through contact investigation. The broad purpose of this project, proposed by investigators at the University of Alabama at Birmingham and the State of Alabama Department of Public Health, is to use a behaviorally focused education that has been proven effective to enhance the accuracy and efficiency of the contact investigation process. The behaviorally focused intervention will focus on enabling public health workers to understand and implement a recently published model of TB transmission. The efficacy of this TB transmission model has been demonstrated by using computer algorithms; however, we endeavor to measure the clinical effectiveness of the TB contact priority model in field application. To achieve this goal, the following specific aims will be accomplished: 1) Enhance the current State of Alabama Department of Public Health contact investigation protocols and computer modules by using the statistically validated models of TB transmission; 2) Modify and re-implement an effective behaviorally focused education intervention to enable TB field workers to understand and implement the TB contact priority model and use the computer modules; 3) Conduct a formal evaluation of the a) behaviorally focused education intervention, b) usefulness of the newly developed computer modules, and c) the clinical effectiveness of the field implementation of the TB contact priority model; and 4) Disseminate and share results with other TB control programs seeking to improve contact investigation and provide more efficient TB disease control.

**Grant:** 1R01HL075026-01A1  
**Program Director:** REYNOLDS, HERBERT Y  
**Principal Investigator:** WOLTERS, PAUL BS  
**Title:** Mast Cells and the Host Response in the Lung  
**Institution:** UNIVERSITY OF CALIFORNIA SAN FRANCISCO SAN FRANCISCO, CA  
**Project Period:** 2004/07/02-2008/06/30

DESCRIPTION (provided by applicant): The long-term objective of our research is to determine the mechanisms by which mast cells regulate the host response to bacterial lung infections and to be able to modify how mast cells coordinate this response in ways that benefit the host. Recently, we made the discovery that the mast cell protease dipeptidyl peptidase I (DPPI) contributes to death of the host from septic peritonitis and that it appears to do so by regulating levels of mast cell IL-6. This suggests that mast cell DPPI, or other mast cell proteins regulated by DPPI, modify the host response to bacterial infection in ways that harm the host. Our central hypothesis is that: Mast cell proteases and cytokines coordinate lung defense against bacterial infections. While coordinating this defense, some of these mediators protect the host and improve survival while others harm the host and worsen survival. Specific aims are: #1. To determine the mechanism by which mast cell DPPI modulates the host response and survival from bacterial lung infections. This will be accomplished by applying a new method for creating mast cell-specific knockout mice and determining the physiologic mechanism for survival following inoculation with *Klebsiella pneumoniae*. #2. To define mechanisms by which mast cell proteases regulate cytokine levels during bacterial infections. We will test whether mast cell proteases hydrolyze cytokines by incubating cytokines with purified DPPI, tryptase or chymase and identifying cleavage products. Protease mediated cytokine production will be studied by measuring cytokines released by specific lung cells in response to DPPI, tryptase or chymase. #3. To determine whether mast cell TNF-alpha, IL-6, or IL-10 modulate the host response and survival from bacterial lung infections. We will use mast cell-specific-TNF-alpha, -IL-6, or -IL-10 knockout mice to test if mast cell sources of these cytokines play important roles in host defense. By understanding how specific mast cell mediators regulate host defense, we will gain greater insight into how these cells influence host survival. This knowledge can then be applied to the development of new treatments for bacterial pneumonia that modulate the activity of specific mast cells proteins in ways that improve survival.

**Grant:** 1R01HL075784-01A1  
**Program Director:** BANKS-SCHLEGEL, SUSAN P.  
**Principal Investigator:** SINGH, PRADEEP K BA  
**Title:** Biofilm Detachment Mechanisms in Chronic Lung Infections  
**Institution:** UNIVERSITY OF IOWA IOWA CITY, IA  
**Project Period:** 2004/08/01-2008/07/31

DESCRIPTION (provided by applicant): *P. aeruginosa* biofilms cause many chronic infections including airway infections that afflict bronchiectasis patients, endotracheal tube colonization that leads to ventilator associated pneumonia, medical device infections, and others. In biofilms, bacteria live in matrix-encased group structures, and this growth mode produces physiologic changes that cause marked antibiotic resistance. In spite of the importance of biofilms in disease, no medical treatments have yet been developed to disrupt them. Interestingly, biofilm bacteria themselves possess active mechanisms to separate from biofilms; this process is known as detachment. Because detached bacteria regain sensitivity to killing, this process may be useful therapeutically. The goal of this work is to investigate a potential mechanism of *P. aeruginosa* biofilm detachment, and to study the pathological consequences of detachment using an airway biofilm infection model. We have discovered a *P. aeruginosa* variant strain with a hyper-detachment phenotype. This strain overproduces the bio-surfactant, rhamnolipid, which is required for its accelerated biofilm detachment. The following aims are proposed: 1. What is the mechanism of rhamnolipid-mediated biofilm detachment? We will examine the types of rhamnolipids produced by the wild type and variant strain, and use two independent methods to determine if rhamnolipids can, by themselves, cause biofilm detachment. Microarray analysis will be used to find other genes that may be needed for detachment. 2. What are the consequences of biofilm detachment in vivo? Using our airway biofilm infection model and isogenic hyper and hypo-detaching strains, we will investigate the potential risks of inducing detachment and determine if detachment improves clearance of the organisms by the host. 3. Will biofilm detachment in vivo increase the efficacy of antibiotic treatment? Using non-detaching strains, infections in the airway biofilm model are extremely resistant to treatment. In vitro, biofilm detachment restores antibiotic sensitivity. We will investigate whether detachment increases the efficacy of antibiotic treatment in vivo. These studies will model a treatment strategy that combines traditional antibiotics with induced biofilm detachment.



**Grant:** 1R01HL076790-01  
**Program Director:** BANKS-SCHLEGEL, SUSAN P.  
**Principal Investigator:** KOLLER, BEVERLY H PHD PATHOBIOLOGY  
**Title:** Role of Onzin, a defensin like molecule, in lung disease  
**Institution:** UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC  
HILL  
**Project Period:** 2004/06/14-2008/05/31

DESCRIPTION (provided by applicant): Airways are protected from invading microorganisms by a highly efficient innate immune system. One defense mechanism available to the lung is the production of airway surface liquid and protective mucus, which ensnares viral and bacterial particles. This protective barrier coupled with the action of the ciliated airway cells, expels most foreign particles without engaging resident immune cells. In addition, most epithelial cells, including those of the airways, secrete microbicidal proteins into the biofilms that separate them from the external environment. One well-characterized family of antimicrobial peptides produced by mammals are the defensins. These are small cationic peptides capable of directly killing both gram positive and gram-negative bacteria as well as fungi and some enveloped viruses. They are also found in neutrophils where they are believed to be essential for non-oxidative killing of ingested microbes. The importance of this family of molecules is underscored by the fact that, not only have these genes been identified in all mammalian species studied, but it is likely that with more than 40 defensins in the human genome, there has been selection for redundancy in this system to ensure maximal protection against a broad spectrum of pathogens. More recently, evidence has emerged that suggest that these peptides might have a number of other important functions both in the innate immune response and in the transition of this response to one that engages lymphocytes. They may act both as natural antibiotics and as signaling molecules that activate host cells involved in immune defense and repair. We have identified a novel gene conserved between mouse and man, termed onzin, whose structure and sequence suggest that it is related to defensins. This gene is expressed by both the airway and the intestinal epithelia and is also expressed in high levels in neutrophils. In this application we test the hypothesis that onzin represents a novel defensive pathway that has a role in the innate immune response of the airways

**Grant:** 1R01HL077431-01  
**Program Director:** HARABIN, ANDREA L.  
**Principal Investigator:** HUNNINGHAKE, GARY W MD BIOLOGY NEC:BIOLOGY  
NEC-UNSPEC  
**Title:** Modulation of Acute Lung Injury  
**Institution:** UNIVERSITY OF IOWA IOWA CITY, IA  
**Project Period:** 2004/09/15-2008/06/30

**DESCRIPTION** (provided by applicant): The overall focus of this grant is acute lung injury and multiple organ failure in bacterial sepsis. Our hypothesis is that a pre-existing viral infection can alter the outcome of sepsis and increase lung injury. We have already shown that a preceding adenoviral infection markedly increases the severity of sepsis in an animal model. We also made the novel observation that a preceding adenoviral infection triggers a resistance (tolerance) to tumor necrosis factor (TNF). The animals exposed to adenovirus are resistant to the effects of TNF( but they make TNF( in normal amounts in response to lipopolysaccharide (LPS). This was true, both with the wild type virus and with an adenoviral vector (deleted in the known adenovirus immunosuppressive proteins E1 and E3). Retained TNF( production after LPS stimulation makes the adenovirus-induced tolerance distinct from LPS tolerance (which blocks subsequent TNF( production). The presence of TNF( tolerance is important because excessive TNF( in the non-resistant state is an important mediator of tissue injury. On the other hand, TNF( and responsiveness to TNF( are essential for optimal clearance of bacterial infections. These observations suggest that the development of TNF( tolerance after a viral infection may contribute to increased incidence, and impaired resolution, of bacterial infections. A second novel finding, suggested by the preliminary data, is that a preceding adenoviral infection interferes with NF(B signaling and decreases production of anti-apoptotic proteins. This may contribute to the increased severity of sepsis by increasing tissue injury. We feel that TNF( tolerance and alterations in survival pathways are important components of virus-induced immunosuppression that can lead to increased severity of sepsis. Our specific aims to evaluate this hypothesis are as follows: 1. Determine the effects of prior exposure to adenovirus on bacterial sepsis and acute lung injury. 2. Determine the effects of prior exposure to adenovirus on TNF( receptor signaling and survival pathways. 3. Determine the mechanisms leading to TNF( tolerance after exposure to adenovirus. These studies may provide important clues to understand the well-described clinical effects of some viruses on subsequent bacterial infections.

**Grant:** 1R01HL078638-01  
**Program Director:** HASAN, AHMED A.K.  
**Principal Investigator:** OWEN, WHYTE G PHD BIOCHEMISTRY  
**Title:** Thrombosis Propensity Determinants of Inflammation  
**Institution:** MAYO CLINIC COLL OF MEDICINE, ROCHESTER, MN  
ROCHESTER  
**Project Period:** 2004/09/24-2008/08/31

**DESCRIPTION** (provided by applicant): The goal of the proposal is understanding the link between inflammation associated with low-grade bacterial infections and thrombotic processes. The underlying hypothesis is that acute or chronic low-grade infection primes platelets and thrombin-generating pathways to increase propensity for thrombosis. The hypothesis will be approached by measuring responses, in vivo and in vitro, of platelets, the clotting system and the vascular anticoagulant system, to acute and chronic doses of endotoxin from Gram-negative bacteria. The studies will take advantage of mice with genetic deficiency in the phylogenetically conserved receptor (toll-like-receptor-4) required for initiating innate immunity to endotoxin of Gram-negative bacteria. Measurements will include intravascular thrombin generation and inhibition and platelet microaggregation in vivo. In addition a family of in vitro assays of platelet physiology and pharmacology, leukocyte interactions and thrombin generation in blood in conjunction with measurement of inflammatory cytokines will provide information about changes in threshold, gain and amplitude of thrombogenic responses. The unique aspect of this proposal is the ability to distinguish acute, non-genomic actions of endotoxin on platelets through the innate immune response, from longer term consequences of changes in gene transcription. Effects of chronic low grade bacterial infection will be studied in humans undergoing initial therapy for advanced bacterial periodontal disease. Blood samples will be obtained from these patients before and after successful treatment, which does not involve pharmacotherapy. These blood samples will be assayed in parallel with those from the mice. This application is highly responsive to the RFA, because it focuses on translational research at the interface of thrombosis and inflammation. The toll family receptors of innate immunity are well characterized for defense against bacteria. We will extend this characterization by defining the mechanism by which these receptors influence thrombotic propensity.

**Grant:** 1R01HL080311-01A1  
**Program Director:** PEAVY, HANNAH H  
**Principal Investigator:** FU, LI M MD  
**Title:** Functional Genomics Study and Database for Tuberculosis  
**Institution:** PACIFIC TUBERCULOSIS/CANCER RES ORG PASADENA, CA  
**Project Period:** 2004/08/12-2007/07/31

DESCRIPTION (provided by applicant): The functional genomics of Mycobacterium tuberculosis will be studied and a database will be constructed for both scientific and clinical applications. Representing a new endeavor in microbiology and genomics, this project is important at this time when multidrug-resistant tuberculosis is increasingly a public-health threat and scientists are seeking new drugs with novel mechanisms of action. In light of the tremendous impact of the microarray technology on genomics, the database will store the microarray gene expression data engendered under various designed experimental conditions as well as provides functional annotations of genes based on expression and regulation profiling. M. tuberculosis clinical isolates both drug-sensitive and -resistant that meet experimental criteria will be obtained. A web-based SQL Server relational database will be developed to implement the functional genomics database, providing query and analysis capabilities via a web-based graphical interface. Each gene in the database will be annotated by its expression characteristics, co-regulated genes and associated regulated pathways or networks, and its clinical significance, if appropriate. Furthermore, the database links each gene to major bioinformatics and genomics databases to produce an integrated retrieved report. All functional genomics data and analyses will be placed in the public domain. Working synergistically with other federally funded resource centers, the database is designed to allow other researchers to deposit microarray data, conduct data analysis, and obtain program code for making in-house systems. In this project, a set of differential and coordinated genome-wide gene expression studies will be performed to explore drug targets, drug resistance, and biology. Important anti-tubercular drugs and promising new drug candidates will be assessed using drug-challenged gene expression studies to induce drug-specific gene-expression patterns resulting from drug action. Cell biology will be investigated using synchronized M. tuberculosis culture based on in vitro induced non-replicating persistence so that cycle-dependent genes and pertinent regulatory mechanisms will be identified and gene expression accompanying metabolic reprogramming that occurs during shift from non-replicating to replicating states will be studied. These studies will uncover many co-regulated families of genes and allow the functions of uncharacterized genes to be deduced based on co-expression with genes of known function. Combining cluster analysis, search of cis-regulatory elements upstream of regulons, and use of transcription factor databases will unravel gene regulatory networks and enable inferences about biological pathways and discovery of novel drug targets. Important regulatory genes identified will be subjected to further analysis for confirming their regulatory roles using knockout strains. Partial drug resistance and bacterial persistence, which are two important clinical circumstances often encountered in tuberculosis, will be analyzed using functional-genomics studies. The potential value of the proposed methods has been demonstrated and advantages over previous technology been recognized. Research results will advance molecular biological knowledge and benefit public health management in tuberculosis.

**Grant:** 1R01HL080312-01  
**Program Director:** PEAVY, HANNAH H  
**Principal Investigator:** BEHAR, SAMUEL M MD  
**Title:** Treatment of tuberculosis with immunomodulators  
**Institution:** BRIGHAM AND WOMEN'S HOSPITAL BOSTON, MA  
**Project Period:** 2004/08/16-2009/07/31

DESCRIPTION (provided by applicant): Tuberculosis is the leading causes of death from infectious disease in the world, and the global situation has considerably worsened because of the emergence of multidrug-resistant strains of *Mycobacterium tuberculosis* (Mtb). This R01 grant application proposes to develop the immunomodulatory glycolipid alpha-galactosylceramide (alphaGalCer) as a treatment for tuberculosis. Our experiments have shown that mice treated with alphaGalCer following inoculation with Mtb survive longer compared to vehicle treated mice. The main action of alphaGalCer is the activation of immunoregulatory CD1d-restricted iNKT cells, which can in turn stimulate both innate and adaptive immunity. This grant proposal will address fundamental questions about the biology of iNKT cells and investigate ways to take advantage of their immunomodulatory effects following activation by alphaGalCer. By defining the pathways that are activated downstream of iNKT cells, we hope to develop a more rational strategy to exploit the immunomodulatory properties of alphaGalCer. It has potential use in three therapeutic arenas. First, its relatively non-selective immunomodulatory properties and potential to provide protection against a variety of pathogens make it a candidate as a biodefense drug for post-exposure prophylaxis. Second, it could be used in combination with traditional antibiotics in the treatment of infectious disease including multidrug resistant Mtb. Third, it could be used as an adjuvant for vaccines. The murine model is an excellent system to investigate the effect of alphaGalCer on Mtb infection since both CD1d and NKT cells are conserved structurally and functionally between mice and humans. Aim 1 will determine the cellular and molecular pathways activated by alphaGalCer that protect mice from tuberculosis after inoculation with Mtb. Since alphaGalCer has been reported to have adjuvant-like properties, in Aim 2, we will administer alphaGalCer concurrently with anti-mycobacterial subunit or DNA vaccines to determine whether such a combination is synergistic in its ability to induce protective immunity following aerosol challenge with Mtb. Aim 3 will determine whether alphaGalCer can protect mice from an aerosol challenge of Mtb using a post-exposure or treatment model. Finally, in Aim 4 we will derive Mtb-specific CD 1d-restricted T cells from infected mice. This comprehensive program on the study of the role of CD1d and NKT cells in response to tuberculosis includes innovative directions that may have applications for the treatment or prevention of tuberculosis, increase the understanding of NKT cells, pulmonary immunity and have implications for the immunomodulation of other infectious diseases.

**Grant:** 1R01HL080972-01  
**Program Director:** TWERY, MICHAEL  
**Principal Investigator:** OPP, MARK R PHD ZOOLOGY  
**Title:** Sleep, Cytokines and Infection  
**Institution:** UNIVERSITY OF MICHIGAN AT ANN ARBOR ANN ARBOR, MI  
**Project Period:** 2004/09/27-2008/06/30

DESCRIPTION (provided by applicant): Infection negatively impacts mental health. Sick individuals become lethargic, experience cognitive deficits and malaise, and lose interest in social contact and other usual daily activities. Prominent among the changes in CNS processes during infection are alterations in sleep. Cytokines, such as interleukin (IL)-1, tumor necrosis factor (TNF), and IL-6 are upregulated during infection. Two lines of evidence suggest that infection-induced alterations in sleep are mediated by actions of these cytokines in brain. First, numerous studies indicate IL-1, TNF, and IL-6 regulate/modulate physiological sleep in the absence of immune challenge. Second, experimental models for which alterations in sleep have been determined are associated with increases in these same cytokines. The involvement of IL-1, TNF, and IL-6 in the regulation of sleep, and the alterations in sleep that occur during infections in which these cytokines are upregulated, have led to suggestions that infection-induced alterations of sleep are mediated by cytokines in brain. Although plausible, and based on empirical evidence, studies to directly test this hypothesis have not been conducted. The fundamental goal of this project is to determine how acute infections alter sleep. To achieve this goal we will use a clinically relevant murine model of infection, sepsis induced by cecal ligation and puncture. We propose experiments that focus on cytokines (IL-1, TNF, IL-6) as mediators of infection-induced alterations in sleep. We will: 1) determine the extent infection alters sleep and the impact of prior sleep loss on responses to infection; 2) quantify alterations in cytokine mRNA and protein in brain during infection; and 3) answer the question "Does interfering with cytokine actions in brain impact infection-induced alterations in sleep?" IL-1, TNF, and IL-6 have been the subject of intense investigation with respect to their peripheral roles in sepsis, and are known central regulators/modulators of sleep. As such, there is a strong conceptual framework within which to investigate the mechanistic relationships between sleep and sepsis, and mediators implicated in both processes. We present preliminary data that demonstrate long-term alterations in CNS function following acute peripheral infection. We demonstrate our ability to determine multiple facets of sleep-wake behavior of mice and to target cytokine systems in brain. Successful completion of the proposed studies will provide information critical to understanding how infection impacts CNS function, as evidenced by alterations in sleep.

**Grant:** 1R21HL077460-01  
**Program Director:** REYNOLDS, HERBERT Y  
**Principal Investigator:** DRAKE, WONDER P. MD  
**Title:** Molecular analysis of frozen sarcoidosis granulomas  
**Institution:** VANDERBILT UNIVERSITY NASHVILLE, TN  
**Project Period:** 2004/07/09-2006/06/30

DESCRIPTION (provided by applicant): Sarcoidosis is a multisystem granulomatous disease of unknown etiology that has worldwide prevalence. In the United States, it most commonly affects African Americans who are less than 40 years of age. The pathologic, epidemiologic, and immunologic features of sarcoidosis are similar to Mycobacterium infections, particularly tuberculosis. In preliminary studies, polymerase chain reaction (PCR) analysis revealed sequences corresponding to Mycobacterium species in 15 of 25 paraffin-embedded granulomas from sarcoidosis patients local to Nashville, TN, but none of 25 control specimens from the same locale ( $p < 0.00002$ , chi square). This analysis was expanded to include frozen specimens from other regions of the United States, in which mycobacterial nucleic acid was amplified from 50% of frozen sarcoidosis specimens and none of control tissues ( $p < 0.016$ , Fisher's exact test). These findings lead to the hypothesis that sarcoidosis is an immunologic response in a genetically susceptible host to mycobacterial infection, involving a novel Mycobacterium which is closely related to Mycobacterium tuberculosis. The proposed research will test this hypothesis by (i) evaluating sarcoidosis specimens for the presence of immunodominant genes associated with virulence in mycobacterial infections, and (ii) comparing antigen specific T-cell responses in sarcoidosis patients with normal healthy controls, and patients with M. tuberculosis infection. This work will contribute important new information about the etiology of sarcoidosis and establish a strong experimental framework for ongoing studies of the immunopathogenesis of this disease.

**Grant:** 1R21HL077462-01  
**Program Director:** REYNOLDS, HERBERT Y  
**Principal Investigator:** SCHWANDER, STEPHAN K PHD  
**Title:** Pathogen Specific Immunity in Sarcoidosis  
**Institution:** UNIV OF MED/DENT NJ NEWARK NEWARK, NJ  
**Project Period:** 2004/07/01-2006/06/30

DESCRIPTION (provided by applicant): Since its initial description 125 years ago, sarcoidosis continues to be a "challenging" disease. Its etiology remains unknown. Discovering the etiology of sarcoidosis remains a major goal with important implications regarding treatment, predicting outcome, as well as determining approaches for preventive measures. Immunological responses and granulomatous tissue formation characterizing sarcoidosis are similar to those observed in a variety of infectious diseases. However, the nature of the specific antigen(s), which putatively trigger the inflammatory response in sarcoidosis, remains elusive. Occurrence of sarcoidosis in spatially related clusters, and household and health care settings strongly support person-to-person transmission of an infectious agent as one of the potential causes of this disease. Sarcoidosis has been associated with a variety of infectious agents, none of which can be cultured. *Propionibacterium acne* (P. acne) and *M.tuberculosis* (Mtb) are the most commonly identifiable infectious pathogens by PCR-based methods and considered to be associated with the development of this disease. Immunological studies in sarcoidosis have focused largely on the assessment of constitutive, immune responses and the description of the phenotypes of blood and lung cells in patients and control subjects. In this proposal we will utilize memory immune responses as search tools for the 'immunological imprints' from P. acne or Mtb exposure. Peripheral blood mononuclear cells and bronchoalveolar cells will be compared from patients with stage II and/or stage III sarcoidosis and from healthy control subjects. We will study by ELISPOT assay: (1) frequencies of pathogen-specific IFN- $\gamma$ - and IL-10-producing cells, and (2) utilizing P. acne- or Mtb-infected autologous monocytes and alveolar macrophages as target cells frequencies of pathogen-specific granzyme B-releasing cytotoxic T lymphocytes and natural killer cells. Finally, we will test the feasibility of identifying by DNA micro array, pathogen specific, transcriptional host gene expression profiles in P. acne- and Mtb-stimulated blood cells from healthy control subjects and patients with active sarcoidosis and to compare these with gene expression profiles from autologous, unstimulated in situ lung cells. Our studies will address the role of P. acne and Mtb in the etiology of sarcoidosis and will also serve as a basis or model for future work involving other possible infectious or non-infectious pathogens/antigens for the development of sarcoidosis.



**Grant:** 1R21HL077581-01  
**Program Director:** REYNOLDS, HERBERT Y  
**Principal Investigator:** TILLEY, STEPHEN L MD  
**Title:** Role of propionibacteria in sarcoidosis  
**Institution:** UNIVERSITY OF NORTH CAROLINA CHAPEL CHAPEL HILL, NC  
HILL  
**Project Period:** 2004/09/01-2006/06/30

DESCRIPTION (provided by applicant): The objective of this proposal is to determine if Propionibacteria are etiologically important to the development of sarcoidosis in the United States (Aim 1), and to develop a mouse model of sarcoidosis using this bacterium (Aim 2). Several lines of evidence suggest that this anaerobic bacteria may be involved in the pathogenesis of disease. First Propionibacteria have been identified by culture and PCR in a high percentage of sarcoid lymph nodes from Japanese and European patients, and studies with in situ molecular probes detected Propionibacteria within sarcoid granulomas. Second, antibiotics effective against this organism showed remarkable efficacy in a small group of patients with cutaneous sarcooidosis. Finally, animals exposed to this bacterium develop granulomatous inflammation similar to sarcoidosis. In Aim 1 we will look for evidence of Propionibacteria by performing quantitative PCR, with probes specific for this organism, on DNA from granulomas obtained by laser capture microscopy from parffin-embedded samples. We will also look for Propionibacteria by in situ hybridization. Finally, we will attempt to culture the organism from tissue obtained from patients undergoing diagnostic evaluation. Cultured bacteria will be characterized from patients with acute-resolving and chronic-persistent disease to see if the bacterial factors contribute to clinical phenotype. Cultured bacteria will also be used for inoculation in mice to produce an animal model. The initiation, maintenance, and resolution of granulomatous inflammation in mice of several different strains will be assessed following exposure to Propionibacteria. These experiments will allow us to look at host factors as determinants of clinical phenotype. Multiple organs will be evaluated including the lungs, lymph nodes, liver, spleen, heart, brain, and eyes, and skin to determine whether host factors or bacterial factors are important for specific organ involvement. Future directions will include a placebo-controlled trial of tetracyclines for sarcoidosis if Propionibacteria are identified.

**Grant:** 1R21HL079393-01

**Program Director:** BANKS-SCHLEGEL, SUSAN P.

**Principal Investigator:** LORY, STEPHEN PHD  
MICROBIOLOGY:BACTERIOLOG  
Y

**Title:** Virulence inhibitors as candidate therapeutics in CF

**Institution:** HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA

**Project Period:** 2004/09/20-2006/06/30

DESCRIPTION (provided by applicant): The major cause of morbidity and mortality of patients with cystic fibrosis is the chronic respiratory infection caused primarily by an opportunistic pathogen *Pseudomonas aeruginosa*. The bacteria that colonize the respiratory tract of CF patients utilize an armament of virulence factors to overcome the host defense mechanisms directed primarily at polymorphonuclear leukocytes. One such mechanism is production of cytotoxic and anti-phagocytic factors ("effectors"), targeted into the host cells by the type III secretion system (TTSS). The objective of this application is to utilize the new field of chemical genetics to develop small molecules as probes of function of the TTSS. The aims of this project are to utilize a sensitive high throughput bioassay (TTSS dependent killing of cultured CHO cells) to identify small molecule inhibitors of cytotoxicity, and further define the mechanism of inhibition of the specific stages of TTSS expression, effector secretion from *P. aeruginosa* and their translocation into the mammalian cell. The active compounds that interfere with the action of TTSS will be screened for activity in *P. aeruginosa* from a variety of clinical sources, with emphasis on mucoid and non-mucoid CF isolates. Furthermore, for each compound or family of compounds, we will identify the specific component of the TTSS, which is the target of inhibition. There is little doubt that there is an urgent need to develop new classes of antimicrobials active against *P. aeruginosa* during all stages of respiratory track colonization of CF patients. An additional outcome of this project will be the identification of groups of small molecules and the establishment of structure activity relationships among them such that they may serve as lead compounds for the development of therapeutic agents against *P. aeruginosa* by inhibiting a key virulence mechanism. This project therefore can lay the foundation for a new research direction in the field of TTSS, leading to a better understanding of this important mechanism of effector targeting, which is currently not possible through the more traditional use of mutants in the TTSS components.

**Grant:** 1R21HL079448-01  
**Program Director:** NOEL, PATRICIA  
**Principal Investigator:** KLINE, JOEL N MD  
**Title:** Early Life Exposure to Microbial Products and Asthma  
**Institution:** UNIVERSITY OF IOWA IOWA CITY, IA  
**Project Period:** 2004/08/01-2006/07/31

**DESCRIPTION** (provided by applicant): Asthma has increased in prevalence and severity in the industrialized world for the past two decades; it is the most common chronic illness of children and has had a rising mortality rate. The "hygiene hypothesis" proposes that reduced early-life exposures to microbes and microbial products are responsible for this epidemic. According to this paradigm, microbial exposures inhibit the development of Th2-skewed responses to environmental allergens, putatively through the induction of Th1 and/or regulatory-type responses. The epidemiological data supporting this hypothesis are compelling, if correlative: children raised in a farm environment (especially with significant exposure to livestock), with increased numbers of older siblings or early daycare attendance (and thus exposed to childhood infections at an earlier age), or with early life exposure to pets have a reduced risk of developing asthma and atopic disorders. The hygiene hypothesis has been difficult to directly test in humans; it remains important to identify the pathways that promote immune tolerance among microbe-exposed children. Understanding the mechanisms of this tolerance will aid development of novel tolerance-inducing therapeutic approaches, and thus reduce the burden, of asthma and atopy. One candidate for inducing tolerance is endotoxin; endotoxin levels in environmental dust correlate with reduced risk of atopy in some studies. Nevertheless, endotoxin inhalation has not been shown to protect against atopic disease in humans; indeed, much evidence points to a deleterious effect of endotoxin in asthma. A second candidate is bacterial (CpG-rich) DNA. We have previously demonstrated, using murine models, that oligonucleotides containing CpG-centered sequence motifs (CpG ODN) are highly effective in both preventing and reversing atopic airway disease. These compounds can suppress allergen-specific immune (Th2-type) responses and induce Th1-type as well as regulatory (IL-10) cytokine responses. CpG-ODN mimics the immunostimulatory effects of native bacterial DNA. Like endotoxin, bacterial DNA is recognized by a toll-like receptor (TLR-9) and potently activates the innate immune system. We suggest that exposure to bacterial DNA may mediate protection against atopy. Thus, we propose to test the hygiene hypothesis, and to compare the modulatory effects of endotoxin and CpG-rich DNA. The overarching hypothesis driving these studies is that early-life exposure to microbial products promotes tolerance and reduces later susceptibility to developing atopic disorders such as asthma. Inhalation of endotoxin and bacterial DNA may influence response to inhaled allergens through different mediators and pathways; these effects may be additive, synergistic, or even antagonistic. We propose to address these hypotheses in the following Aims: Aim 1: To test the hypothesis that early-life exposure to microbial products (bacterial DNA or endotoxin) reduces later susceptibility to developing atopic disorders. Question 1A: Does early life exposure to microbial products alter the pulmonary inflammatory milieu? Question 1B: Does early life exposure to microbial products alter later susceptibility to developing atopic asthma? Question 1C: Is early-life exposure to antigen as well as microbial products required for antigen-specific tolerance? Aim 2: To test the hypothesis that early-life exposure to microbial products promotes immune tolerance by inducing a regulatory cell population. Question 2A: What mediators promote immune tolerance induced by early life exposure to microbial products? Question 2B: What cells are responsible for immune tolerance induced by early life exposure to microbial products?

**Grant:** 1R21HL080313-01  
**Program Director:** PEAVY, HANNAH H  
**Principal Investigator:** ACTOR, JEFFREY K PHD  
**Title:** Regulation of Cortisol by Mycobacterial Glycolipid TDM  
**Institution:** UNIVERSITY OF TEXAS HLTH SCI CTR HOUSTON, TX  
HOUSTON  
**Project Period:** 2004/08/23-2006/07/31

**DESCRIPTION** (provided by applicant): The observation of glucocorticoid modulation during tuberculosis infection has been known for many years, however the relationship between glucocorticoid immunomodulation and destructive pathological and granulomatous responses to mycobacterial antigens remains poorly understood. Indeed, relative amounts of both systemic and tissue specific cortisol may be crucial in susceptibility to tuberculosis, and therefore play a defining role in ensuing pathology. C57BL/6 mice demonstrate a rapid decrease in serum cortisol levels during initiation of granulomatous response during acute experimental infection with virulent *Mycobacterium tuberculosis*. Investigators have actively pursued agents that mimic immunopathology associated with tuberculosis. One such agent; the natural mycobacterial glycolipid trehalose 3,6'dimycolate (TDM) has been researched extensively as a mediator of biological events and granulomatous responses during disease. We have further identified mycobacterial TDM as able to influence systemic serum cortisol levels. This is the first observation of mediation of a systemic glucocorticoid to a mycobacterial glycolipid, and warrants further investigation. Our preliminary findings indicate a need to explore the influence of cortisol on initial events involved in the immunoregulation and immunopathology due to mycobacterial glycolipid TDM. The goal of this application is therefore to examine the relationship between immune- responses induced by mycobacterial glycolipid and associated concurrent imbalances in both serum and tissue glucocorticoids (cortisol) during development of the pulmonary granulomatous response. We request that this proposal be considered for R21 funding directed towards exploratory research into applications of physioneuroimmunology towards understanding the basis of immunoregulated disease pathology during tuberculosis infection.

**Grant:** 1R01MH069116-01A1  
**Program Director:** KOPNISKY, KATHY L.  
**Principal Investigator:** ZINK, M C DVM VET  
MEDICINE:MICROBIOLOGY  
**Title:** Neuroprotective Effects of Minocycline in Lentiviral Inf  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 2004/04/01-2009/03/31

DESCRIPTION (provided by applicant): HIV CNS disease is consistently associated with infiltration and activation of macrophages/microglia, enhanced production of proinflammatory cytokines, increased expression of proapoptotic and neurotoxic mediators, and neuronal loss. A number of neuroprotective therapeutics are being examined, but no single agent has emerged as the solution to the inflammatory and neurodegenerative effects of HIV in the CNS. The recent identification of the tetracycline derivative, minocycline, as a potent anti-inflammatory and neuroprotective drug that also inhibits HIV replication in macrophages, microglial cells, and astrocytes demands the examination of this readily available generic drug as a neuroprotective agent in HIV infection. We have developed an accelerated, consistent SIV/macaque model (SIV-AC) of HIV CNS disease in which over 90% of infected animals develop encephalitis with neurodegeneration as evidenced by increased expression of B-APP and B-amyloid and evidence of neuronal degeneration/apoptosis in the CSF and brain. This model recapitulates the acute, asymptomatic, and terminal characteristics of HIV infection in humans on a highly reproducible time schedule. Our recent studies using this model have demonstrated that the development of SIV encephalitis coincides with an imbalance between the antiapoptotic ERK signaling pathways and the proapoptotic JNK and p38 signaling pathways, representing a failure to maintain a homeostatic balance in the CNS. Our hypothesis is that minocycline will play a dual neuroprotective role in SIV-infected macaques: a) by inhibiting pathologic activation of p38 thus reestablishing a balance between pro- and antiapoptotic pathways, and b) by inhibiting SIV replication and hence the production of viral neurotoxic proteins in the CNS. This application proposes integrated in vivo and in vitro studies to examine the mechanisms by which minocycline exerts its palliative effects on the CNS. In Aim 1 we propose to measure the effects of minocycline on virus replication and on the development of CNS inflammatory and neurodegenerative changes in SIV-infected macaques. In Aims 2 and 3 we will identify the mechanism(s) by which minocycline protects against neurotoxicity and suppresses SIV/HIV replication in macrophages, microglia and astrocytes. These mechanistic studies are important given the potential of minocycline to act not only as a neuroprotective agent but also as a viral suppressive agent in the CNS.

**Grant:** 1R01MH070039-01A1  
**Program Director:** BRADY, LINDA S.  
**Principal Investigator:** GOUAUX, ERIC PHD  
**Title:** Structure and Function of Neurotransmitter Transporters  
**Institution:** COLUMBIA UNIVERSITY HEALTH SCIENCES NEW YORK, NY  
**Project Period:** 2004/07/01-2009/04/30

DESCRIPTION (provided by applicant): Transporters for neutral and anionic amino acids play key roles in human physiology and are active in organs as diverse as the kidney and brain. In the central nervous system, glutamate mediates the majority of fast excitatory signaling, a form of neuron-neuron communication that is essential to the development and maintenance of the nervous system. A fundamental component of glutamate-mediated signaling is the removal of glutamate from the synaptic cleft, following an excitatory stimulus, by sodium-dependent, high affinity glutamate transporters in neurons and other cells, such as glial cells. At the present time, there is no atomic resolution structural information on a glutamate transporter, which greatly hampers our understanding of their architecture and mechanism of action. In this application I propose to determine the structure of a bacterial protein that has significant sequence identity to the eukaryotic glutamate transporters, using x-ray crystallography. Furthermore, I plan to determine the functional behavior of the bacterial homolog, and to test structure-based mechanisms of transporter function. In addition, by using the crystal structure(s) of the bacterial protein as a guide, I will create a homology model of selected eukaryotic transporters and, together with previously determined structure and function information, this will place structure and function relationships of the eukaryotic transporters in an atomic-resolution, three-dimensional context. Taken together, the proposed research will substantially further our understanding of both eukaryotic and prokaryotic glutamate transporters, and, because they are related to transporters of dicarboxylic acids and of neutral amino acids, our knowledge of these secondary transporters will be increased as well. Lastly, because glutamatergic signaling is pervasive in the human nervous system, the structure of the bacterial transporter, along with the homology models of the eukaryotic transporters, should facilitate the design of new molecules that may have therapeutic potential.

**Grant:** 1R03MH070603-01A1  
**Program Director:** SIEBER, BETH-ANNE  
**Principal Investigator:** ELDER, GREGORY A MD  
**Title:** Creation of a Nestin Lineage Stem Cell Ablation System  
**Institution:** MOUNT SINAI SCHOOL OF MEDICINE OF NYU NEW YORK, NY  
**Project Period:** 2004/09/30-2006/06/30

DESCRIPTION (provided by applicant): Neurogenesis occurs in adult brain in discrete regions in creatures as diverse as mammals, amphibians, birds and reptiles. Its occurrence in adult mammals is firmly established in the hippocampus and the subventricular zone surrounding the lateral ventricles. Neurogenesis in adult brain may be regarded as one aspect of neural plasticity and altered hippocampal neurogenesis may play a pathophysiological role in disease states and in the therapeutic action of certain drugs. For example administration of all classes of antidepressants tested to date enhance hippocampal neurogenesis by stimulating the proliferation of neural progenitor cells and tricyclic antidepressants can reverse stress-induced reductions in neural progenitor proliferation in the hippocampus. Hippocampal neurogenesis may also be altered in other disease states including cerebral ischemia, seizures and Alzheimer's disease. Yet despite its undoubted existence and possible role in disease states the functional significance of hippocampal neurogenesis in adult brain remains unclear. One major limitation in identifying functional roles for neurogenesis in the adult remains the lack of methods to selectively perturb neurogenesis. Here we propose to develop a system to selectively target a cytotoxic agent, the diphtheria toxin A chain (DT-A) to adult CNS stem cells using a recently described system that relies on transgene regulation by lac repressor. This system will allow an inducible ablation of nestin lineage neural stem cells and thus of neurogenesis at any age in adult animals and will permit the effects of altered neurogenesis in the adult to be assessed independent of secondary affects on the non-progenitor population.

**Grant:** 1R01NR008545-01A1  
**Program Director:** HARE, MARTHA L  
**Principal Investigator:** HASSELGREN, PER-OLOF J  
**Title:** C/EBP, atrogin-1, and muscle wasting  
**Institution:** BETH ISRAEL DEACONESS MEDICAL CENTER BOSTON, MA  
**Project Period:** 2004/09/30-2009/08/31

**DESCRIPTION** (provided by applicant): Sepsis and other catabolic conditions are characterized by muscle wasting, mainly reflecting increased degradation of myofibrillar proteins. The role of muscle wasting for morbidity and mortality in catabolic conditions is not fully understood. Glucocorticoids, together with proinflammatory cytokines, are major mediators of muscle proteolysis. Muscle wasting is at least in part caused by ubiquitin-proteasome-dependent proteolysis. The gene expression of several components of the ubiquitin-proteasome pathway, including ubiquitin ligases, is increased in catabolic muscle but mechanisms responsible for gene activation in muscle wasting is poorly understood. In particular, the role of the transcription factor C/EBP and nuclear co-factors p300/CBP for regulation of genes in the ubiquitin-proteasome pathway has not been defined. The specific aims are to test the hypotheses that 1) increased muscle proteolysis contributes to mortality during sepsis; 2) glucocorticoids and cytokines upregulate the expression and activity of C/EBP in skeletal muscle; 3) glucocorticoids and cytokines upregulate the expression and activity of p300/CBP in skeletal muscle; 4) glucocorticoid- and cytokine-dependent C/EBP activation and C/EBP-p300/CBP interaction activate the ubiquitin ligase atrogin-1; 5) the glucocorticoid- and cytokine-induced C/EBP-atrogin-1 gene activation cascade is at least in part responsible for muscle wasting. The role of increased muscle proteolysis for mortality in sepsis will be tested by creating transgenic mice overexpressing 11 $\beta$ -HSDt or 11 $\beta$ -HSD2 selectively in skeletal muscle, thereby creating conditions with high and low muscle corticosterone levels, respectively. In other experiments, rats are treated with dexamethasone and/or the glucocorticoid receptor antagonist RU38486 followed by determination of expression and activity of C/EBP, p300/CBP and atrogin-1. In other experiments, cultured L6 myotubes are treated with dexamethasone and proinflammatory cytokines. DNA binding activity of C/EBP beta and delta is determined by EMSA and supershift analysis. Protein and gene expression of C/EBP beta and delta, p300/CBP, and atrogin-1, is determined by Western blotting and real-time PCR, respectively. Coimmunoprecipitation is used to examine protein-protein interaction between p300/CBP and C/EBP transcription factors. The role of C/EBP and p300/CBP in glucocorticoid- and cytokine-induced activation of atrogin-1 and protein degradation is determined by transfecting myocytes with expression plasmids for C/EBPbeta or delta and p300/CBP or antisense oligonucleotides. The project is important because it will test the role of muscle wasting for mortality in sepsis and will link the activation of a transcription factor and nuclear co-factors to the activation of a gene in the ubiquitin-proteasome pathway and muscle proteolysis.



**Grant:** 2R01NS026310-17  
**Program Director:** NUNN, MICHAEL  
**Principal Investigator:** KIM, KWANG S MD PEDIATRICS:PEDIATRICS-UNSPEC  
**Title:** E. coli invasion of brain endothelial cells  
**Institution:** JOHNS HOPKINS UNIVERSITY BALTIMORE, MD  
**Project Period:** 1988/03/01-2009/06/30

DESCRIPTION (provided by applicant): The mortality and morbidity associated with neonatal bacterial meningitis remain significant despite advances in antimicrobial chemotherapy and supportive care. A major contributing factor is the incomplete understanding of the pathogenesis of this disease. E. coli is the most common gram-negative organism that causes meningitis during the neonatal period. Most cases of E. coli meningitis in newborns develop as a result of hematogenous spread, but it is not completely understood how circulating E. coli cross the blood-brain barrier. We have developed an in vitro model of the blood-brain barrier with human brain microvascular endothelial cells (HBMEC) and an infant rat model of experimental hematogenous E. coli meningitis, which mimics human E. coli meningitis (e.g., hematogenous infection of the meninges). Using these in vitro and in vivo systems, we have shown that E. coli K1 traverses the blood-brain barrier without altering the integrity of HBMEC monolayers and inducing a change in blood-brain barrier permeability. During the previous funding period, we showed that E. coli K1 invades HBMEC through a zipper-like mechanism and transmigrates through an enclosed vacuole without intracellular multiplication. We identified that several E. coli K1 determinants contribute to HBMEC invasion in vitro and crossing of the blood-brain barrier in vivo and some of the E. coli proteins interact with the specific receptors present on HBMEC. We also showed that the K1 capsule has a novel role in E. coli traversal of the blood-brain barrier as live bacteria. Based on these findings, we would like to examine the following specific aims. 1.To continue to characterize microbial determinants contributing to E. coli K1 invasion of HBMEC in vitro and traversal of the blood-brain barrier in vivo. 2.To examine the mechanisms involved in E. coli K1 invasion of HBMEC by identifying and characterizing HBMEC receptors as well as by determining host cell signal transduction pathways. 3.To determine the role of K1 capsule in E. coli trafficking in HBMEC. Further understanding and characterization of E. coli K1-HBMEC interactions should allow us to develop novel strategies to prevent this serious infection.

**Grant:** 2R01NS034235-08  
**Program Director:** NUNN, MICHAEL  
**Principal Investigator:** MARRA, CHRISTINA M MD CLINICAL MEDICAL  
SCIENCES, OTHER  
**Title:** Persistence of CNS T. pallidum in HIV Infection  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 1996/07/01-2008/05/31

**DESCRIPTION** (provided by applicant): The US and Europe are currently experiencing outbreaks of syphilis in men who have sex with men, many of whom are HIV-infected. This is of particular concern because neurosyphilis may be more common in HIV-infected individuals. Our neurosyphilis studies began in 1996. The overall goal of our original proposal was to test the hypothesis that HIV infection impairs clearance of *Treponema pallidum* from the cerebrospinal fluid (CSF). The results of our studies support this hypothesis. Specifically, individuals with more pronounced HIV-mediated immunosuppression are more likely to have neurosyphilis, and normalization of CSF-Venereal Disease Research Laboratory (VDRL) reactivity after treatment for neurosyphilis is significantly less likely in HIV-infected individuals, particularly those with advanced immunosuppression. In our competing renewal, we focused on predicting neurosyphilis and its treatment response. In the last 15 months, we have made substantial progress in addressing our previous Aims. We have shown that the CSF cellular phenotype can be used to support a diagnosis of neurosyphilis in HIV-infected patients, have additional preliminary data in support of the association between greater genetic diversity of blood T. pallidum and neurosyphilis, and have identified new markers of neurosyphilis treatment response. As of Dec 1, 2003, we have enrolled and obtained CSF from 470 subjects with syphilis, and 91 have had at least one follow-up lumbar puncture after neurosyphilis treatment. Approximately three-quarters of our subjects are also HIV-infected. In this competing renewal application, we continue to focus on clinically and pathogenetically important questions regarding neurosyphilis and HIV. The Specific Aims are: 1) Identify measures that predict a high likelihood of neurosyphilis in HIV-infected and -uninfected individuals; 2) Distinguish CSF pleocytosis due to T. pallidum infection from CSF pleocytosis due to HIV infection; 3) Determine factors associated with response to neurosyphilis therapy in HIV-infected and -uninfected individuals. Our ongoing study is the largest investigation of neurosyphilis in many decades, and is the only study with sufficient power to address the effect of concomitant HIV infection on development of neurosyphilis and the response to neurosyphilis therapy in both HIV-infected and -uninfected individuals.

**Grant:** 2R01NS040730-05  
**Program Director:** NUNN, MICHAEL  
**Principal Investigator:** KIELIAN, TAMMY L PHD MICROBIOLOGY, OTHER  
**Title:** Pathogenesis of Brain Abscess  
**Institution:** UNIVERSITY OF ARKANSAS MED SCIS LTL LITTLE ROCK, AR  
ROCK  
**Project Period:** 2001/05/01-2008/11/30

**DESCRIPTION** (provided by applicant): Brain abscesses represent an important medical problem despite recent advances made in detection and therapy. Because of the emergence of multi-drug resistant strains and the ubiquitous nature of bacteria, these CNS infections are likely to persist. The size of a developing abscess normally extends well beyond the original site of infection leading to damage of surrounding normal brain parenchyma. This finding suggests that the CNS antibacterial response is not down regulated in an efficient manner, resulting in chronic inflammation and large abscess lesions. They propose that a balance exists between sufficient and over-compensatory responses to *S. aureus* in the CNS, which dictates the outcome of brain abscess development; therefore, therapies aimed at attenuating chronic CNS inflammation subsequent to effective bacterial neutralization may result in smaller abscesses and subsequent improvements in cognitive and neurological functions. The objective of the proposed work is to examine the influences of minocycline and PPAR-gamma agonists on the pathogenesis of brain abscess development. Recently, these compounds have been found to exhibit neuroprotective effects in several models of CNS disease; however, their roles in regulating CNS infectious disease has not yet been examined. To address this objective, the following Specific Aims will be addressed: (I) to evaluate the dose- and time-dependent effects of PPAR-gamma agonists and minocycline on *S. aureus*-induced brain abscess development; (II) to investigate the effects of PPAR-gamma agonists and minocycline on cell migration and neuronal cell death induced by *S. aureus*-stimulated microglia; and (III) to examine the mechanism(s) responsible for impaired neutrophil infiltration into brain abscesses of CXCR2 KO mice and the potential effects of PPAR-gamma agonists and minocycline in the CNS compartment. In addition to its anti-inflammatory properties, the bacteriostatic activity of minocycline may augment its effects on brain abscess development. The potential multifactorial effects of these compounds suggest that they may be more efficacious compared to traditional therapies developed to counteract a single pathway in CNS diseases. These experiments should provide meaningful insights into how minocycline and PPAR-gamma agonists influence brain abscess development and will reveal whether their ability to modulate non-infectious CNS conditions extends to infectious diseases.

**Grant:** 1R01NS048952-01  
**Program Director:** NUNN, MICHAEL  
**Principal Investigator:** PHILIPP, MARIO T  
**Title:** Lyme neuroborreliosis pathogenesis in the rhesus monkey  
**Institution:** TULANE UNIVERSITY OF LOUISIANA NEW ORLEANS, LA  
**Project Period:** 2004/07/01-2009/06/30

**DESCRIPTION** (provided by applicant): The broad, long-term objective of this proposal is to understand the pathogenesis of Lyme neuroborreliosis of the central nervous system (CNS). Inflammation in the CNS is thought to play a primary role in the pathogenesis of neurodegenerative diseases such as Alzheimer's disease (AD) and the AIDS-dementia complex (ADC). Lyme borreliosis (LB), caused by the spirochete *Borrelia burgdorferi*, is primarily an inflammatory disease, and neuroborreliosis, i.e. LB of the nervous system, is a mild neurodegenerative disease. The central hypothesis to be explored in this grant proposal is that 1) *B. burgdorferi* spirochetes that have accessed the CNS can cause inflammation and 2) this inflammatory response may lead to neural injury, and ultimately loss of neural cells. Limited loss of these cells is viewed as a determinant of the neurologic impairment seen in Lyme neuroborreliosis. The secondary hypothesis to be examined is that spirochetal lipoproteins can induce, in part or in toto, the inflammatory effects of *B. burgdorferi*. More specifically, the cytokines IL-6, TNF-alpha, and IL-1beta, which have been implicated as possible effectors/mediators of neurodegeneration in AD and ADC, are elicited in the CNS by *B. burgdorferi* or its lipoproteins, and can mediate glial/neuronal loss. A corollary of our secondary hypotheses is that the innate immune response, in the form of a response to pathogen-associated molecular patterns (in this case lipoproteins) exerts via toll-like receptors (TLR) some or all of the inflammatory effects of *B. burgdorferi*. The following specific aims are proposed, using the rhesus macaque, the only animal model of LB of the CNS: SA-1: Experiments in vivo. SA-1a: assessment of inflammation and glial/neuronal loss in rhesus after intracerebral stereotaxic inoculations with live spirochetes and lipoproteins. Local cytokine secretion will be assessed by confocal microscopy using a novel procedure whereby cytokines are stained intracellularly with appropriate antibodies in freshly fixed brain slices treated ex vivo with Brefeldin A. The cell phenotype also will be identified, TLR expression determined, and glial/neuronal apoptosis verified in situ by the TUNEL assay. Spirochetal antigens will be localized with anti-lipoprotein antibodies. Neurons will be counted using image analysis software, and numbers compared with control animals; SA-1b: as with SA-1a, but following natural infections (by tick) of rhesus with *B. burgdorferi*; SA-1c: Archival fixed samples of Bb-infected rhesus brain and meninges will be assessed for inflammatory infiltrates and glial/neuronal apoptosis. Neurons will be counted differentially as above. SA-2: Experiments in vitro. SA-2a will assess the production of IL-1beta, TNF-alpha and IL-6 in single-cell-type and mixed primary cultures of rhesus glia stimulated with live *B. burgdorferi* and lipoprotein. The human neuroblastoma cell lines SK-N-SH and SH-SY5Y also will be employed, alone and in combination with rhesus glia, as well as rhesus neuronal-glial primary cultures; SA-2b will assess the role of these cytokines in eliciting glial/neuronal apoptosis; SA-2c will assess the expression and role of TLR in mediating cytokine production and apoptosis in the different cell combinations.

**Grant:** 1R03NS050782-01

**Program Director:** HEEMSKERK, JILL E

**Principal Investigator:** TSE-DINH, YUK-CHING      PHD  
BIOCHEMISTRY:BIOCHEMISTR  
Y-OTHER

**Title:** Development of a HTS system for topoisomerase targets

**Institution:** NEW YORK MEDICAL COLLEGE      VALHALLA, NY

**Project Period:** 2004/09/30-2005/08/31

DESCRIPTION (provided by applicant): DNA topoisomerases are important targets for therapy against potential viral and bacterial pathogens. The removal of transcription-driven positive and negative supercoils in DNA is a major function of topoisomerases, including poxvirus topoisomerase I. The trapping of covalent intermediates complexed with cleaved DNA formed by topoisomerases during removal of transcription-driven supercoiling can lead to immediate loss of infectivity. However, cell based viral or bacterial count assays cannot distinguish between such topoisomerase-targeting mechanism and other modes of anti-infectivity. In vitro assays with purified enzyme and DNA substrates do not provide twin domains of positive and negative supercoiling that are the sites of action of topoisomerases involved in transcription in vivo. An E. coli based assay would place the target topoisomerase at sites of transcription-driven supercoils, allowing identification of agents that would trap the target topoisomerases during active transcription. The concurrent use of a target topoisomerase active site mutant can immediately confirm if the positive result is due to trapping of covalent topoisomerase complex. Based on our previous work on the effect of vaccinia virus topoisomerase I expression on E. coli DNA supercoiling, we plan to develop a system suitable for high-throughput screening of chemical libraries for potential small pox therapeutic agents that will act by trapping poxvirus topoisomerase complexed with cleaved viral DNA. The screening system should also be applicable for targeting topoisomerases in pathogenic bacteria relevant for biodefense.

**Grant:** 1R03NS050811-01  
**Program Director:** HEEMSKERK, JILL E  
**Principal Investigator:** BURGESS, RICHARD R PHD  
**Title:** LRET-based HT Screening for Inhibitors of Transcription  
**Institution:** UNIVERSITY OF WISCONSIN MADISON MADISON, WI  
**Project Period:** 2004/09/30-2005/08/31

DESCRIPTION (provided by applicant): The binding of sigma factors to core RNA polymerase is essential for specific initiation of transcription in eubacteria and is thus critical for cell growth. Since the responsible protein-binding regions are highly conserved among all eubacteria but differ significantly from eukaryotic RNA polymerases, it is a promising target for drug discovery. A homogeneous assay for sigma-binding to RNA polymerase (E. coli) based on luminescence resonance energy transfer (LRET) has been developed in our lab using an europium-labeled sigma70 and IC5-labeled fragment of the beta-prime subunit of RNA polymerase (amino acid residues 100-309). Inhibition of sigma binding was measured by the loss of LRET through a decrease in IC5-emission. The technical advances offered by LRET resulted in a very robust assay suitable for a high-throughput screening and was successfully used to screen a crude natural product library. We would like to refine this assay, to develop appropriate counter-screens to minimize the number of false positives, and to develop further methods for characterizing the confirmed hits. Then we will use these assays to screen much larger libraries and hopefully to identify and characterize chemical compounds that might represent lead compounds for new antibiotic drug development.

**Grant:** 1R03NS050814-01  
**Program Director:** HEEMSKERK, JILL E  
**Principal Investigator:** PERRIMON, NORBERT  
**Title:** High-throughput screen for factors in Listeria infection  
**Institution:** HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA  
**Project Period:** 2004/09/30-2005/08/31

**DESCRIPTION** (provided by applicant): The turn of the twentieth century has witnessed the continued appearance of new pathogens as well as the development of antibiotic resistance and re-emergence of old pathogens. Although the war on infectious diseases was once declared over, numerous factors have conspired to make bacterial pathogens a persistent threat. Despite the enormous burden that bacterial pathogens impose on human health, there is relatively little understanding of host-pathogen interactions. In understanding the molecular details of such interactions, we will gain insight into eukaryotic cell biology. In addition, this knowledge is of vast practical importance, as it is directly relevant to disease prevention and treatment. The goal of this grant proposal is to conduct high-throughput screens using RNA interference (RNAi) to identify host factors involved in the entry and replication of the intracellular pathogen *Listeria monocytogenes* in *Drosophila* S2 cells. These screens will be complemented with compound libraries' screens to identify small molecules that affect bacteria uptake, the ability of bacteria to establish their intracellular niche, or the ability of bacteria to survive and divide. By combining the two approaches and developing high throughput assays that survey host-pathogen interactions between *Listeria* and *Drosophila* cells, we anticipate identifying genes that are important in bacterial entry, reorganization of vesicle transport, cytoskeletal rearrangements, microbial killing, bacterial nutrition, host cell death, and immune recognition.

**Grant:** 1R21NS047216-01  
**Program Director:** MAMOUNAS, LAURA  
**Principal Investigator:** BUTOWT, RAFAL PHD  
**Title:** The Role of p75 Receptor in Tetanus Toxin Transport  
**Institution:** UNIVERSITY OF NEVADA RENO RENO, NV  
**Project Period:** 2004/01/01-2005/12/31

DESCRIPTION (provided by applicant): Bacterial neurotoxins (botulinum and tetanus) produced by genus *Clostridium* are among the most toxic natural substances known. They share many structural and functional similarities, however their sites of action within the nervous system are quite different. Botulinum toxin (BoT) is acting in the periphery, whereas tetanus toxin (TeT) acts in the central nervous system (CNS). It is believed that this phenomenon is due to high affinity binding to different specific protein receptors present at presynaptic membranes. Likely, TeT binds to a receptor which under normal conditions enters the endocytotic pathway and travels along axons while BoT does not. Despite 30 years of research on Clostridial neurotoxins this aspect of their biology remains largely unknown. No protein receptor or non-protein binding molecule has been identified to date which could explain extensive neuronal trafficking of TeT but lack of this feature for homologous BoT molecule(s). The proposed project focuses on a molecular and biochemical analysis of axonal trafficking of TeT. On the base of preliminary results and indirect data existing in the literature, it is proposed that the common neurotrophin p75 receptor may be used as a binding protein and/or axonal carrier for TeT molecules. This project includes a set of experiments to test this hypothesis. Possible interactions between TeT and p75 receptor will be examined in vitro and in vivo under different conditions and in different neuronal cells. Finally, the role of the p75 receptor in TeT axonal trafficking will be functionally probed using neuronal lines in culture with down-regulation of p75 expression as well as in p75 knock-out mouse models. These experiments will clarify the potential role of p75 in TeT neuronal transport. It could be a major advancement in our understanding of neuronal intoxication and may participate in extending knowledge about poorly understood endogenous retrograde axonal pathways used by neurons for delivery of different trophic molecules during development. Highly efficient TeT trafficking pathway through binding to p75 receptor will open an entry gate leading from the periphery to the central nervous system. This may assist in the development of novel modes of delivery of diverse biological agents, including enzymes, analgesics, anesthetic drugs or trophic factors, to the spinal cord and brain stem (Dobrenis et al., 1992; Schneider et al., 2000; Schiavo et al., 2000; Bordet et al., 2001; Kissa et al., 2002).



**Grant:** 1R03NS050811-01  
**Program Director:**  
**Principal Investigator:** BURGESS, RICHARD R PHD  
**Title:** LRET-based HT Screening for Inhibitors of Transcription  
**Institution:** UNIVERSITY OF WISCONSIN MADISON MADISON, WI  
**Project Period:** 2004/09/30-2005/08/31

DESCRIPTION (provided by applicant): The binding of sigma factors to core RNA polymerase is essential for specific initiation of transcription in eubacteria and is thus critical for cell growth. Since the responsible protein-binding regions are highly conserved among all eubacteria but differ significantly from eukaryotic RNA polymerases, it is a promising target for drug discovery. A homogeneous assay for sigma-binding to RNA polymerase (E. coli) based on luminescence resonance energy transfer (LRET) has been developed in our lab using an europium-labeled sigma70 and IC5-labeled fragment of the beta-prime subunit of RNA polymerase (amino acid residues 100-309). Inhibition of sigma binding was measured by the loss of LRET through a decrease in IC5-emission. The technical advances offered by LRET resulted in a very robust assay suitable for a high-throughput screening and was successfully used to screen a crude natural product library. We would like to refine this assay, to develop appropriate counter-screens to minimize the number of false positives, and to develop further methods for characterizing the confirmed hits. Then we will use these assays to screen much larger libraries and hopefully to identify and characterize chemical compounds that might represent lead compounds for new antibiotic drug development.

**Grant:** 1R03NS050814-01  
**Program Director:**  
**Principal Investigator:** PERRIMON, NORBERT  
**Title:** High-throughput screen for factors in Listeria infection  
**Institution:** HARVARD UNIVERSITY (MEDICAL SCHOOL) BOSTON, MA  
**Project Period:** 2004/09/30-2005/08/31

**DESCRIPTION** (provided by applicant): The turn of the twentieth century has witnessed the continued appearance of new pathogens as well as the development of antibiotic resistance and re-emergence of old pathogens. Although the war on infectious diseases was once declared over, numerous factors have conspired to make bacterial pathogens a persistent threat. Despite the enormous burden that bacterial pathogens impose on human health, there is relatively little understanding of host-pathogen interactions. In understanding the molecular details of such interactions, we will gain insight into eukaryotic cell biology. In addition, this knowledge is of vast practical importance, as it is directly relevant to disease prevention and treatment. The goal of this grant proposal is to conduct high-throughput screens using RNA interference (RNAi) to identify host factors involved in the entry and replication of the intracellular pathogen *Listeria monocytogenes* in *Drosophila* S2 cells. These screens will be complemented with compound libraries' screens to identify small molecules that affect bacteria uptake, the ability of bacteria to establish their intracellular niche, or the ability of bacteria to survive and divide. By combining the two approaches and developing high throughput assays that survey host-pathogen interactions between *Listeria* and *Drosophila* cells, we anticipate identifying genes that are important in bacterial entry, reorganization of vesicle transport, cytoskeletal rearrangements, microbial killing, bacterial nutrition, host cell death, and immune recognition.

**Grant:** 1R21NS047216-01  
**Program Director:** MAMOUNAS, LAURA  
**Principal Investigator:** BUTOWT, RAFAL PHD  
**Title:** The Role of p75 Receptor in Tetanus Toxin Transport  
**Institution:** UNIVERSITY OF NEVADA RENO RENO, NV  
**Project Period:** 2004/01/01-2005/12/31

DESCRIPTION (provided by applicant): Bacterial neurotoxins (botulinum and tetanus) produced by genus *Clostridium* are among the most toxic natural substances known. They share many structural and functional similarities, however their sites of action within the nervous system are quite different. Botulinum toxin (BoT) is acting in the periphery, whereas tetanus toxin (TeT) acts in the central nervous system (CNS). It is believed that this phenomenon is due to high affinity binding to different specific protein receptors present at presynaptic membranes. Likely, TeT binds to a receptor which under normal conditions enters the endocytotic pathway and travels along axons while BoT does not. Despite 30 years of research on Clostridial neurotoxins this aspect of their biology remains largely unknown. No protein receptor or non-protein binding molecule has been identified to date which could explain extensive neuronal trafficking of TeT but lack of this feature for homologous BoT molecule(s). The proposed project focuses on a molecular and biochemical analysis of axonal trafficking of TeT. On the base of preliminary results and indirect data existing in the literature, it is proposed that the common neurotrophin p75 receptor may be used as a binding protein and/or axonal carrier for TeT molecules. This project includes a set of experiments to test this hypothesis. Possible interactions between TeT and p75 receptor will be examined in vitro and in vivo under different conditions and in different neuronal cells. Finally, the role of the p75 receptor in TeT axonal trafficking will be functionally probed using neuronal lines in culture with down-regulation of p75 expression as well as in p75 knock-out mouse models. These experiments will clarify the potential role of p75 in TeT neuronal transport. It could be a major advancement in our understanding of neuronal intoxication and may participate in extending knowledge about poorly understood endogenous retrograde axonal pathways used by neurons for delivery of different trophic molecules during development. Highly efficient TeT trafficking pathway through binding to p75 receptor will open an entry gate leading from the periphery to the central nervous system. This may assist in the development of novel modes of delivery of diverse biological agents, including enzymes, analgesics, anesthetic drugs or trophic factors, to the spinal cord and brain stem (Dobrenis et al., 1992; Schneider et al., 2000; Schiavo et al., 2000; Bordet et al., 2001; Kissa et al., 2002).

**Grant:** 2R01RR013601-06A2  
**Program Director:** O'NEILL, RAYMOND R.  
**Principal Investigator:** CHEN, ZHENG W MD BIOMEDICAL RESEARCH  
**Title:** Role of CD8 T cells in immunity to tuberculosis  
**Institution:** UNIVERSITY OF ILLINOIS AT CHICAGO CHICAGO, IL  
**Project Period:** 1998/06/01-2009/07/31

DESCRIPTION (provided by applicant): Tuberculosis remains an important life-threatening disease and has recently been declared to be a global health emergency by the World Health Organization. While human CD4 T cells play a crucial role in immune protection against *M. tuberculosis* infection, other T cell populations are poorly characterized for their roles in immunity to tuberculosis. We have recently demonstrated that memory responses of mycobacterium-specific CD8 T cells after *M. tuberculosis* infection coincided with protection against fatal tuberculosis in BCG-vaccinated monkeys, and that antibody-mediated depletion of CD8 T cells in monkeys with protective memory resulted in the development of severe forms of tuberculosis following *M. tuberculosis* re-infection by aerosol. Based on these results, we hypothesize that mycobacterium-specific CD8 T cells are an important component of immunity against tuberculosis. In testing this hypothesis, we will answer immune mechanistic questions involving CD8 T cell-mediated anti-tuberculosis immunity. We will: I. Determine the role of CD8 T cells in resistance to primary *M. tuberculosis* infection. II. Determine the role of CD8 T cells in adaptive immunity to *M. tuberculosis* re-infection and reactivation tuberculosis. A. Determine the role of CD8 T cells in adaptive immunity against *M. tuberculosis* re-infection. B. Determine the role of CD8 T cells in controlling reactivation tuberculosis in normal monkeys and SIVmac-infected macaques. III. Determine if vaccine-elicited CD8 T cell responses can confer some protection against tuberculosis in immune competent and SHIV-infected monkeys. A. Examine vaccine-elicited CD8 T cell responses and determine if such immune responses can confer some degree of protection against *M. tuberculosis* infection in normal monkeys. B. Determine if vaccine-elicited CD8 T cell responses can contribute to immune protection against tuberculosis in SHIV-89.6P-infected monkeys.

**Grant:** 2R03TW001319-04  
**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:** 2000/01/01-2007/07/31

**DESCRIPTION** (provided by applicant) This research will be done primarily in St. Petersburg, Russia at Petersburg Nuclear Physics Institute in collaboration with Vladislav Lanzov as an extension of NIH grant Number R01GM32335. 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 R01 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 GM32335-21 (recently funded) is to understand how RecA function is autoregulated. *Pseudomonas aeruginosa*, an important human pathogen, is the source of a RecA protein that we will investigate to further this aim. We propose to continue a productive collaboration that has been extending our understanding of RecA autoregulation through an analysis of the *Pseudomonas aeruginosa* RecA protein. Work to date has provided numerous mechanistic and structural insights that we will build on. Together with the laboratory of Dr. Vladislava Lanzov in St. Petersburg, Russia, we will further explore the biochemistry of the *P. aeruginosa* RecA protein, and define the enzymatic differences between it and the *E. coli* RecA. We have pinpointed likely regions of the protein responsible for autoregulation and for certain other functional differences between the RecA proteins. The new work should help test key features of 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:** 1R03TW006235-01A1  
**Program Director:** MICHELS, KATHLEEN M  
**Principal Investigator:** HITTI, JANE E AB  
**Title:** M. genitalium: behavioral and reproductive correlates  
**Institution:** UNIVERSITY OF WASHINGTON SEATTLE, WA  
**Project Period:** 2003/12/15-2006/11/30

DESCRIPTION (provided by applicant): Mycoplasma genitalium is a sexually transmitted microorganism recently identified from vaginal and cervical specimens using PCR technology, with a prevalence of 3-5%. This microbe is capable of invading the upper genital tract, and thus could plausibly be a cause of preterm birth and other pregnancy complications. However, the role of M. genitalium in preterm birth has not been systematically evaluated. We propose to examine the behavioral, infectious and reproductive correlates of M. genitalium infection among pregnant women in Lima, Peru. Our study design will take advantage of an on-going case-control study of infectious causes of preterm birth. 750 preterm cases and 750 controls will be selected from deliveries at the Instituto Materno Perinatal, a large tertiary referral center in Lima. Cervical swabs will be obtained after delivery for M. genitalium by PCR, as well as Chlamydia trachomatis and Neisseria gonorrhoeae. We will also evaluate vaginal Trichomonas and bacterial vaginosis. Demographic and behavioral information will be collected by standardized interview. The study has the following specific aims: 1. To examine the associations between M.genitalium infection and other STD with spontaneous preterm birth. 2. To examine sexual history and behavioral characteristics as risk factors for M. genitalium and other STD in pregnancy among Peruvian women. This research will be conducted primarily in Lima, Peru in collaboration with Pedro Garcia, MD, as an extension of NIH grant HD 41682.

**Grant:** 1R03TW006262-01A1  
**Program Director:** SINA, BARBARA J  
**Principal Investigator:** SCHOOLNIK, GARY K MD  
**Title:** Biofilm Population Structure in the Gangetic Delta  
**Institution:** STANFORD UNIVERSITY STANFORD, CA  
**Project Period:** 2003/12/15-2006/11/30

**DESCRIPTION** (provided by applicant) The research project summarized below, a collaboration between Drs. Schoolnik and Md. Sirajul Islam, will be performed mainly in Bangladesh at the International Center for Diarrhoeal Diseases Research (ICDDR,B) as an extension of NIH grant RO1 AI43422. Observational field studies performed in Bangladesh and elsewhere indicate that *Vibrio cholerae*, the agent of Asiatic cholera, is a normal member of natural aquatic habitats. In the rivers and estuaries that compose the Gangetic Delta, *V. cholerae* is believed to be associated with phyto- and zoo- plankton and to reside in mixed-species biofilms on a variety of surfaces. One such surface, the chitinous exoskeletons of copepods, can be degraded by *V. cholerae* chitinases, thus providing a source of nitrogen, carbon and energy. Other surfaces, including sand particles in the benthic layer of the estuary, are nutrient-poor. Biofilm bacteria on non-nutritive surfaces of this kind must obtain nutrients from the water column. In turn, the nutrient content and chemical features of the water column are dramatically affected by the monsoon seasonal cycle. As a result, the movement of *V. cholerae* between these sites in its aquatic reservoir and the human host is believed to be determined, in part, by the effects of the monsoon climate on the physicochemical features of this habitat. To begin to understand how these seasonal changes affect *V. cholerae*-containing mixed-species biofilms in the Ganges Delta, Drs. Islam and Schoolnik recently completed a one year pilot study (supported as a supplement to the parent grant) that showed the following: (1) *V. cholerae* resides in mixed species biofilms that form on non-nutritive plastic surfaces submerged in the Ganges Delta near Matlab, Bangladesh; and (2), the species composition of the biofilm changes during the year. These observations and the importance and abundance of chitin as a nutritive surface led to the following hypothesis: the population structure of *V. cholerae*-containing mixed-species biofilms varies as a function of the surface substratum (chitin compared to non-nutritive surfaces) and season (pre-monsoon, monsoon or post-monsoon). To test this hypothesis, the work proposed here will use a culture-independent, 16S rRNA sequence-based method to determine the population structure of biofilm consortia on chitin or polycarbonate surfaces submerged at the Matlab sampling site during the pre-monsoon, monsoon and post-monsoon periods. The identified species-specific sequences will then be used to design labeled pligonucleotide probes for fluorescence in situ hybridization (FISH) assays; these probes will be used in the last two years of the project to determine the population structure of monthly biofilm samples from chitin and polycarbonate surfaces. These results will be correlated with changes in water chemistry and cholera case rates. The transfer of each component of these key molecular ecology methods to the ICDDR,B--achieved by a training program for Bangladeshi scientists at the ICDDR,B and at Stanford--will significantly enhance the research capacity of this important Center.

**Grant:** 1R03TW006270-01A1  
**Program Director:** SINA, BARBARA J  
**Principal Investigator:** CHEN, TIE MD  
**Title:** Mechanisms of Host Responses in Gonorrhea  
**Institution:** INDIANA UNIV-PURDUE UNIV AT INDIANAPOLIS, IN  
INDIANAPOLIS  
**Project Period:** 2003/12/15-2006/11/30

DESCRIPTION (provided by applicant): *Neisseria gonorrhoeae* (gonococci, GC) cause gonorrhea and pelvic inflammatory disease (PID). Studies show that gonorrhea can facilitate infection of both HIV and Chlamydia trachomatis (CT). The infection results from the ability of the pathogens to adhere to and penetrate host cells. However, little is known about whether the host immune responses play a role in GC infection. To establish infection, bacteria must interact with receptors on host cells. The opacity (Opa) proteins of GC mediate adherence and phagocytosis in epithelial cells and neutrophils in part through interaction with members of the carcinoembryonic antigen family (CEA, CEACAM, or CD66), CEACAM3 (CD66d) and CEACAM1 (CD66a). CEACAM1 is an inhibitory receptor, which mediates negative signals in DT40 B cells. The biological functions of inhibitory receptors are the inhibition of phagocytic ability of cells and proliferation as well as antibody production in B cells. Neutrophils and lymphocytes play a critical role in protection against infectious bacteria. Consistent with our preliminary data that GC inhibits the production of antibodies in human B cells in vitro, local and systemic anti-GC antibody levels in gonorrhea patients with a history of prior infection are low, suggesting that GC might inhibit local host immune responses. In the proposed research, we hypothesize that local immune inhibition mediated by GC infection may be achieved through the following two events: 1). GC binds to neutrophils and inhibits their ability to phagocytose microorganisms. 2). GC enters the host and binds to CEACAM1 on B cells to activate inhibitory pathways, and consequently inhibits lymphocyte proliferation and antibody production. We expect that signal transduction is involved in these events. Therefore, we propose these specific aims to: 1. Determine whether GC inhibits the phagocytic ability of neutrophils. 2. Elucidate how the interaction of CEACAM1 with GC inhibits antibody production. 3. Identify CEACAM3 and CEACAM1-mediated signal transduction pathways following infection with GC. The proposed research will begin to unveil the potential mechanisms of antibody suppression, which consequently play a role in the GC induced immunosuppression during infection. We believe that these studies will uncover some mechanisms of GC infection and will help us to understand how microbial pathogens exploit host cells. This knowledge will allow us to develop novel strategies to combat other infectious diseases.



**Grant:** 1R03TW006324-01A1  
**Program Director:** SINA, BARBARA J  
**Principal Investigator:** SMITH, DANIEL J PHD OTHER FIELDS:GENERAL  
SCIENCE  
**Title:** Muscosal Immunity in Heavily S. Mutans Exposed Children  
**Institution:** FORSYTH INSTITUTE BOSTON, MA  
**Project Period:** 2004/09/01-2006/08/31

**DESCRIPTION** (provided by applicant) Despite considerable knowledge of dental caries pathogenesis, this transmissible infectious disease is still a worldwide public health problem, affecting mainly populations under high Streptococcus mutans challenge. Although use of fluoride has beneficial effect, poor oral hygiene and high sucrose consumption continue to promote early S. mutans infection and disease. Preclinical studies with dental caries vaccines have successfully interfered with S. mutans infection and disease. This approach requires a level of secretory immune maturity sufficient for adequate response to virulence antigen(s) contained within these vaccines before infection. Children normally challenged with S. mutans become infected between 18-36 months of age and form salivary IgA antibody to several S. mutans antigens. However, poorly understood are secretory immune responses to these antigens in children who become colonized much earlier due to heavy challenge. To this end, the NIDCR has requested studies of the relationships of adaptive immunity to S. mutans, degree of infection, and caries development. We propose a prospective study to investigate IgA antibody responses to initial colonizers (S. mitis) and pathogenic bacteria (S. mutans) during the period of initial oral establishment of S. mutans in a population undergoing heavy S. mutans infectious challenge. 160 children will be followed at 6m intervals from 6-12m until 24-30m of age. Clinical examinations will be followed by microbiological exams at each interval to analyze levels of infection by S. mitis and S. mutans. The genetic diversity and stability of S. mutans clones will be measured. Salivary samples will be collected to measure total levels of IgA and levels of IgA antibody to S. mitis/S. mutans secreted/surface-associated antigens. Patterns and extent of IgA responses to these streptococcal strains will be compared to assess relative maturation of secretory immune responses. These data will then be analyzed with respect to the time and intensity of S. mutans infection and development of dental caries. These studies should help to define vaccine approaches targeting children at high risk for dental caries.

**Grant:** 1R03TW006828-01A1  
**Program Director:** KATZ, FLORA  
**Principal Investigator:** SEVERINOV, KONSTANTIN V PHD  
**Title:** Structure-function of RNAP inhibitor microcin J25  
**Institution:** RUTGERS THE ST UNIV OF NJ NEW BRUNSWICK, NJ  
BRUNSWICK  
**Project Period:** 2004/09/01-2007/08/31

**DESCRIPTION** (provided by applicant) This research will be done primarily in University of Tucuman, Argentina in collaboration with Raul Salomon as an extension of NIH grant # R01GM64530. The long-term goal is to understand the structure, function and maturation of 21 amino acid antibiotic Microcin J25 (MccJ25). Our previous work showed that MccJ25, which is produced from a 58 amino acid-long McjA precursor by the action of McjB and McjC proteins targets bacterial RNA polymerase. Further research showed that Mccj25 has a unique structural fold--a threaded lasso. The complementary strengths of the US and Argentinian groups will be combined to address the following specific aims: 1. To establish structure-functional relationships in MccJ25 molecule. Systematic site-specific and random mutagenesis of the MccJ25 moiety of the plasmid-born mcjA gene will be performed. The ability of cells bearing mutant mcjA plasmids to produce mutant MccJ25 in the cultured medium in the presence of a complementary plasmid bearing mcjBC maturation genes and mcjD immunity gene will be established; MccJ25 mutants will be purified and their antibacterial and transcription inhibitory functions will be established by in vivo and in vitro tests, respectively. MccJ25 mutants active against cells resistant to wild-type MccJ25 will also be sought. 2. To establish an in vitro system for MccJ25 maturation. Purified recombinant McjA precursor and McjB and McjC will be prepared and used to find conditions for efficient maturation of McjA into MccJ25. The results of this work will clarify the details of MccJ25 synthesis, structure-function and interaction with its target and thus will allow new insights into the mechanism of transcription. Understanding of MccJ25 maturation may allow to use McjA-based chimeric proteins to construct novel cyclic peptides and or proteins with potentially interesting biological and pharmacological properties.

**Grant:** 1R03TW006833-01

**Program Director:** SINA, BARBARA J

**Principal Investigator:** FLAVELL, RICHARD A PHD  
BIOCHEMISTRY:PROTEIN/AMI  
NO ACID

**Title:** Roles of bacterial flora in endotoxin shock mediated vi\*

**Institution:** YALE UNIVERSITY NEW HAVEN, CT

**Project Period:** 2003/12/15-2006/11/30

DESCRIPTION (provided by applicant) Approximately 20000 people die from septic shock every year alone in this country as an outcome of gram negative bacterial infection. Lipopolysaccharide (LPS) or endotoxin, a structural component of outer cell wall, is a major effector and can activate the innate immune system via Toll-like receptors (TLRs) and lead to multiple organ damage, termed endotoxin shock. Human bodies are cohabitating with commensal bacterial flora. The largest observed is the one in the intestinal tract which is approximately 1 kg of bacteria/adult of weight. These bacterial flora continuously yield large amounts of bacterial products such as LPS. It has been shown that previous exposure to bacterial products change the sensitivity to LPS challenge in vitro and in vivo. Since LPS produced by commensal bacterial flora in the intestine is actually observed in blood circulation in the portal vein in laboratory animals, it is conceivable that commensal bacterial flora simulate the immune system, leading to the change of sensitivity to endotoxin shock via TLRs. We will test if commensal bacterial flora alter TLR signaling and sensitivity to endotoxin shock in vivo, using mutant strains of mice with increased TLR signaling and decreased TLR signaling. This research is performed primarily in the Institute of Microbiology AS CR in the Czech Republic in collaboration with Helena Tlaskalova as an extension of NIH grant # P01 AI36529.

**Grant:** 1R03TW006982-01  
**Program Director:** MCDERMOTT, JEANNE  
**Principal Investigator:** DERETIC, VOJO P PHD MOLECULAR BIOLOGY,  
OTHER  
**Title:** Tuberculosis and Autophagy  
**Institution:** UNIVERSITY OF NEW MEXICO ALBUQUERQUE ALBUQUERQUE, NM  
**Project Period:** 2004/03/01-2007/02/28

DESCRIPTION (provided by applicant): Tuberculosis is the most common opportunistic infection in patients with AIDS. A synergism between the etiologic agent of tuberculosis, *Mycobacterium tuberculosis*, and HIV is widely acknowledged. *M. tuberculosis* is a facultative intracellular organism that infects macrophages residing in a specialized phagosome that does not follow the default pathway of maturation into the phagolysosome. Our central objective is to find new methods to counteract or overcome the intracellular trafficking block imposed by mycobacteria. We have evidence that, in cells incubated under conditions that stimulate the autophagic pathway, mycobacterium-containing phagosomes acidify and acquire markers of lysosomal compartments resulting in a loss of bacterial viability. Thus, autophagy, which can be pharmacologically induced, is the topic of the present proposal in the context of inhibiting *M. tuberculosis* intracellular growth. Our main goal is to study the role of autophagy in phagosomal biogenesis and killing of intracellular *M. tuberculosis* in order to develop future strategies for tuberculosis treatment. Since our preliminary results indicate that induction of autophagy, by amino acid deprivation or by pharmacological means, results in an override of the maturation block imposed by mycobacteria, we will further prove these relationships by assessing the effect of LC3 and Rab24 overexpression, as these proteins are either known or are suspected to control important stages in the autophagic pathway. Specifically, we will determine the role of these proteins on maturation of the mycobacterial phagosome specialized autophagy regulators that may control the PI3K hVPS34 activity on the properties of mycobacterium-containing phagosomes and determine whether they promote antimycobacterial activity. The proposed experimental approaches will allow us to prove autophagy can counteract the intracellular trafficking block imposed by mycobacteria and find new modalities for eliminating pathogenic mycobacteria. Thus, defining the molecular mechanism by which autophagy affects intracellular mycobacteria will allow us to find new strategies to control the persistence, latency, and transmission of tuberculosis.